

Date and Time: 1/13/14 1:00 PMProtocol: glycerol stock

Reagents: \_\_\_\_\_

40% glycerol/LB broth 9/1/13  
liquid cultureLab Technicians(s) involved: Serra Tackett

## Procedure (with applicable notes):

Used liquid culture from 1/9/13① NISSLE + C4MR 3x① NISSLE 3x500 ml broth500 ML liq culture.

## Results:

Location of product: -80°C Plasmid Stock Box 1Label on product: NISSLE or NISSLE + C4MR, date, STEnd Notes/Comments: #from bacteria in fridge for 3+ mo.

## NEXT STEP:

Continued on back? Yes ☐; No ☒

Date and Time: 1/13/14Lab Technicians(s) involved: Steven Van AlstineProtocol: Gel electrophoresisReagents: BglI PCR product (3)BglI Plasmid, BglI Part 26x Loading dye (from PCR box)1 kb ladder (from PCR box) Agarose 1xTAE buffer

## Procedure (with applicable notes):

1% agarose gel made and loaded with samples as follows:  
 (Tubes in boxes are numbered arbitrarily) Each well contained 2  $\mu$ l of DNA sample and .4 loading dye  
 Gel run at 100V

| Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6  | Lane 7 | Lane 8 |
|--------|--------|--------|--------|--------|---------|--------|--------|
| Ladder | Blank  | PCR1   | PCR2   | PCR3   | Plasmid | Part 1 | Part 2 |

Gel started at 9:52 PM

Ended at 10:29 PM

Stored in plastic bag in 4°

## Results:

see phone Steve's phone.  
 All travel same distance (PCR1, PCR2, PCR3 all were around 700 bps (which is what we want since BglI is 729 bps)  
 Part 1 also was around 700 bps  
 Plasmid and Part two traveled same distance but indicating size smaller than .5 kb.

Location of product: 4°Label on product: BglI test gelEnd Notes/Comments: PCR1, PCR2, PCR3, and Part 1 all had are right size.

## NEXT STEP:

Analysis then PCR on PCR1, PCR2, PCR3, and Part 1Continued on back? Yes ☐; No ☒

Date and Time: 1/14/14Lab Technicians(s) involved: Steven V., AislingProtocol: PCRJacob TiedtReagents: TopShot PCR 2x Master-mixDEPC water, BglI primersPCR1, PCR2, PCR3, Part 1Labeled by box taken from

Procedure (with applicable notes):

Post PCR product "template" {  
 PCR1 = \ 12  $\mu$ L Master-mix DEPC H<sub>2</sub>O taken from BioBrick Assembly kit  
 PCR2 = X 1  $\mu$ L rev primer Used BglI "BioBrick" primers  
 PCR3 = ~~■~~ 1  $\mu$ L forward primer  
 Part 1 = ~~■~~  $\Delta$  + 2  $\mu$ L template  
 + 16  $\mu$ L DEPC H<sub>2</sub>O  
 25  $\mu$ L total  
 Used Lambda Biotec One-Shot PCR kit used vial #1  
 Ran PCR on thermocycler protocol "Lambda" for 2 hrs. Started at 8:05 PM ended at 10:07 PM  
 Products immediately run through agarose gel (see Gel Electrophoresis 1/14/14)

Results:

See analysis of Gel Electrophoresis of same day

Location of product: PCR tubes in green pipette rack labeled "BglI PCR product Ende" in 4°Label on product: "BglI PCR product PCR1, PCR2X, PCR3, Part 1"End Notes/Comments: When Mastermix had loading dye included so none added for electrophoresis.

NEXT STEP:

Gel electrophoresis analysis

Continued on back? Yes ☐; No ☒

Date and Time: 1/14/14Lab Technicians(s) involved: Steven Van AlstineProtocol: Gel ElectrophoresisJacob TodatReagents: Agarose1x TAE BufferGel Green 10000xPCR Product from PCR on 1/14/141 kb ladder

## Procedure (with applicable notes):

Made 50 mL 1% agarose gel. <sup>2ul</sup> Loaded it with 1 kb ladder and <sup>2ul</sup> PCR product from 1/14/14 in map below.

| Line 1      | Line 2 | Line 3 | Line 4 | Line 5 | Line 6 | Line 7 | Line 8 |
|-------------|--------|--------|--------|--------|--------|--------|--------|
| 1 kb ladder | Blank  | PCR 1  | Blank  | PCR 2  | Blank  | PCR 3  | Pos 1  |

Gel ran ~~at~~ at 100V starting at 10:30 PM  
ended at 11:30 PM.

Since ~~PCR kit~~ PCR kit used (One-Shot PCR kit by Lambda Biotek) had loading dye included, no additional loading dye was added.

## Results:

Picture on Jacob Todat's phone (to be emailed to Steven Van Alstine)

PCR Appeared to have failed. Only primers showed up. Bands far below 5 kb band of ladder. Investigate if PCR kit is valid. Since no controls used could be the following: Possible thermocycler issues (wrong temp etc), <sup>(unlikely)</sup> PCR kit bad, primers bad, not be Bgl gene, Possible too low concentration of template.

Location of product: Plastic bag in 4°Label on product: "Bgl PCR product test gel"End Notes/Comments: Needs to be redone with proper controls

## NEXT STEP:

Redo with proper controls

Continued on back? Yes ☐; No ☒



Date and Time: 1/15/14 8pmProtocol: Run PCR products in gelLab Technicians(s) involved: Ryan George  
Steven van AlstineReagents: yesZ PCR product, xynA PCR product1x TAE BufferGelGreen 10,000xAgarose1 kb ladderboth from PCR box

## Procedure (with applicable notes):

Made 50 mL 1% agarose gel.

Loaded gel w/ 1 kb ladder and 2 µL PCR Product

| Ln1         | Ln2  | Ln3  | Ln4  | Ln5  | Ln6  | Ln7  |
|-------------|------|------|------|------|------|------|
| 1 kb ladder | PCR1 | PCR2 | PCR3 | PCR4 | PCR5 | PCR6 |

Gel ran @ 100V start: ~~10:20pm~~ 9:20pm

end: 10:21 pm

## Results:

Picture on Ryan George's iPad (to be emailed to Anna Garvel)

PCR products 2 and 3 (both are xynA) appear to contain the correct DNA when compared to the ladder (about 800 bp)

PCR products 4-6 (all yesZ) appear to contain the correct DNA when compared to the ladder (about 2.0 kb)

PCR product 1 did not produce a band. There is likely no DNA in this sample.

Location of product: corner of GEM PCR Box in freezer (-20°C)Label on product: "xynA uncut PCR product" (blue marker 1-3) "yesZ uncut PCR product" (blue marker 4-6)End Notes/Comments: went as expected except for PCR product 1The 3 BglBis PCR products were previously run in agarose gel.

## NEXT STEP:

Verify correct DNA w/ PCRContinued on back? Yes ☐; No ☒

Date and Time: 1/15/14Lab Technicians(s) involved: Steven VanAlstineProtocol: PCRReagents: Banda Bztech 2x Mastermix vial #1Bgls forward + rev primersBgls BioBrick fwd + rev primersB. subtilis 168 genome prep, Original Bgls 1mMBgls BioBrick, DEPC H<sub>2</sub>O from BioBrick Assembly box

Procedure (with applicable notes):

|   |  |  |
|---|--|--|
| Code: <u>B. subtilis 168 genome: G</u><br><u>Original Bgls 1mM: P</u><br><u>Bgls BioBrick: B</u>  | 12 mL 2x Mastermix<br>1 mL Bgls forward primer<br>1 mL Bgls reverse primer<br>2 mL B. subtilis 168 genome prep<br>+ 9 mL DEPC H <sub>2</sub> O<br><u>25 mL</u> | PCR was run on<br>Protocol "BGLS" on thermocycler<br>for 1 hr <del>40-50</del> min<br>Started at 9:00 PM.<br>ended at 10:00 PM |
| 12 mL 2x Mastermix<br>1 mL <del>Bgls BioBrick</del> fwd primer<br>1 mL Bgls BioBrick rev primer<br>2 mL Bgls BioBrick<br>+ 9 mL DEPC H <sub>2</sub> O<br><u>25 mL</u> | 12 mL 2x Mastermix<br>1 mL Bgls forward primer<br>1 mL Bgls reverse primer<br>2 mL Original Bgls 1mM<br>9 mL DEPC H <sub>2</sub> O<br><u>25 mL</u>             |  |

Results:

See gel electrophoresis analysis on page 7

Location of product: 4<sup>u</sup> in green rack labeledLabel on product: "Bgls PCR product"End Notes/Comments: Need to be run through gel

NEXT STEP:

Run on gel to see if correct genes.Continued on back? Yes ☐; No ☒

Date and Time: 1/16/14Lab Technicians(s) involved: Steven Van AbtineProtocol: Gel electrophoresisReagents: Bgl<sub>1</sub> PCR product made on 1/15/14Agarose, 1X TBE Buffer

## Procedure (with applicable notes):

50 mL 1% Agarose gel made and loaded with ~~PCR~~ Bgl<sub>1</sub> PCR product and 1 kb Ladder. Map of lanes below. 1.5 mL of Ladder added

| Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 |
|--------|--------|--------|--------|--------|--------|--------|--------|
| Ladder | Blank  | P      | Blank  | G      | Blank  | Blank  | B      |

2 mL of PCR product added

P: Original Bgl<sub>1</sub> Indel

G: B. subtilis 168 genome

B: Bgl<sub>1</sub> BioBrick

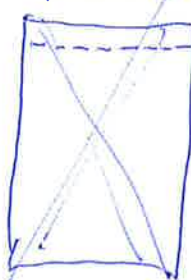
Run at 100 V starting at 9:32 PM

ended at 10:40 PM

## Results:

(Picture on phone)

Schematic



Only band that showed was from the genomic DNA, at a little above 5 kb. Only faint primer dimers for the rest.

Bgl<sub>1</sub> primers work on Genomic DNA not on Original Bgl<sub>1</sub> Indel

Bgl<sub>1</sub> BioBrick primers did not work on bioBrick

Location of product: BiohazardLabel on product: N/AEnd Notes/Comments: Only genomic DNA got PCR primer productInvestigate primers for BioBrick

## NEXT STEP:

Don't have gel extraction kit. Obtain one!

~~Use amplicon from gel and isolate it. Amplify more from the genome or amplicon to get stock of Bgl<sub>1</sub> amplicon.~~ Purify genomic DNA from B. subtilis 168 and explore the efficiency of the primers

Continued on back? Yes ☐; No ☒

Date and Time: 1/18  
 Protocol: Gel Electrophoresis  
 Reagents: Agarose, TAE, Gel Green

Lab Technicians(s) involved: NWM  
 \_\_\_\_\_  
 \_\_\_\_\_

Procedure (with applicable notes):

Made 0.8% agarose gel

Found all instances of yes2 not in a mastermix instock

Used 2uL DNA + 0.4 uL 6x Loading Dye

Loaded 10uL 1kb ladder into lane 1 and the rest of the samples as shown (by box)

Ran gel at 100V starting at 7:15pm

Results: Incomplete

Location of product: none

Label on product: none

End Notes/Comments: Gel failed

NEXT STEP:

Try again

Continued on back? Yes ☐; No ☒

Date and Time: 1/18/14 5pm  
 Protocol: Gel Electrophoresis  
 Reagents: 1 kb ladder, gel made  
 on 1/17/14 (see p 8) various yes2  
 labeled reactants from -20°C, orange  
 loading dye, 1x TAE

Lab Technicians(s) involved: MW/M

Procedure (with applicable notes):

5 uL DNA + 1 uL Loading Dye (6x)  
 10 uL ladder  
 Started running at 5:24 pm at 100 V

ladder 1 kb  
 PCR Box  
 yes2  
 Plasmid Box  
 yes2

Results:

The plasmid box was smaller than .5 kb  
 The others were of ~~various~~ identical or very close length. They were located between the 2k and 3k bands, slightly closer to the 3k side

Location of product: picture on teamspace

Label on product: 2014-1-18 p 9 yes2 Stock Analysis

End Notes/Comments: according to the yes2 registry page, yes2 should be 1996 bp

NEXT STEP:

Investigate extra strand length

Continued on back? Yes ☐; No ☐



Date and Time: 1/18/14

Lab Technicians(s) involved: Steven Van Houten

Protocol: PCR

Ashlynn Wasielewski

Reagents: ~~Bamda Botech 2x Mastermix~~ PCR 2x Mastermix vial #1

Greg Borak

XynA primers (Fwd, Rev)

XynA BioBrick (Fwd, Rev)

B. subtilis 168 genome, XynA part 1, XynA part 2

XynA BioBrick, DEPC H<sub>2</sub>O, XynA PCR product 1, XynA PCR product 2

Procedure (with applicable notes):

|                        |                             |                            |
|------------------------|-----------------------------|----------------------------|
| \ = B. subtilis genome | \ Reagents:                 | Δ reagents                 |
| X = XynA part 1        | 12 mL 2x Mastermix          | 12 mL 2x mastermix         |
| Δ = XynA part 2        | 1 mL Fwd XynA primer        | 1 mL Fwd XynA primer       |
| □ = XynA BioBrick      | 1 mL Reverse XynA primer    | 1 mL Reverse XynA primer   |
| ● = XynA PCR product 1 | 2 mL B. subtilis 168 genome | 2 mL XynA part 2           |
| ■ = XynA PCR product 2 | 9 mL DEPC H <sub>2</sub> O  | 9 mL DEPC H <sub>2</sub> O |
|                        | 25 mL                       | 25 mL                      |
|                        | X Reagents                  | □ reagents                 |
|                        | 12 mL 2x Mastermix          | 12 2x mastermix            |
|                        | 1 mL Fwd XynA primer        | 1 Fwd XynA primer          |
|                        | 1 mL Reverse XynA primer    | 1 Reverse XynA primer      |
|                        | 2 mL XynA part 1            | 2 XynA BioBrick            |
|                        | 9 mL DEPC H <sub>2</sub> O  | 9 DEPC H <sub>2</sub> O    |
|                        | 25 mL                       | 25                         |

cont. →

Results:

See Gel electrophoresis on 1/19/14

Location of product: 4° in green rack

Label on product: XynA PCR Test PCR Product

End Notes/Comments:

NEXT STEP:

Gel electrophoresis

Continued on back? Yes ☒; No ☐

▲ reagents

12  $\mu$ L 2x mastermix  
1  $\mu$ L Fwd Xyn A primer  
1  $\mu$ L Reverse Xyn A primer  
2  $\mu$ L Xyn A PCR product 1  
4  $\mu$ L DEPC H<sub>2</sub>O  
25  $\mu$ L

■ reagents

12  $\mu$ L 2x mastermix  
1  $\mu$ L Fwd Xyn A primer  
1  $\mu$ L Reverse Xyn A primer  
2  $\mu$ L Xyn A PCR product 2  
4  $\mu$ L DEPC H<sub>2</sub>O  

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25  $\mu$ L

Date and Time: 1/19/14

Lab Technicians(s) involved: Steven VanAalst

Protocol: Genome Isolation

Christa Winslow

Reagents: DNeasy Blood &amp; Tissue Kit

B. subtilis subtilis 168 liquid culture

## Procedure (with applicable notes):

B. subtilis subtilis 168 ~~centrifuged~~ down pelleted down in microcentrifuge tube.

AL Buffer was added in first step instead of enzymatic lysis buffer

~~New tubes were~~ New collection tubes were not used for washes since no extra collection tubes available.

DNA was eluted twice for higher yield but lower concentration

## Results:

See Assay by gel electrophoresis  
and PCR

Location of product: -20°

Label on product: B. subtilis subtilis 168 genome prep

End Notes/Comments:

## NEXT STEP:

Assay by gel electrophoresis and PCR

Continued on back? Yes ☐; No ☒

Date and Time: 11/11/14 5:25

Protocol: Gel Electrophoresis

Reagents: PCR products of

(B. Subtilis genome, XynA part 1  
 XynA part 2, XynA Biebrick  
 XynA PCR product 1, XynA PCR product 2)  
 Agarose, TAE buffer, ~~loading dye~~,  
 10,000x gel green, 1Kb ladder

Procedure (with applicable notes):

- \ = B. Subtilis genome
- X = XynA part 1
- Δ = XynA part 2
- = XynA Biebrick
- ▲ = XynA PCR product 1
- = XynA PCR product 2
- made 50 mL 1% agarose gel
- PCR products include loading dye
- 5 μL of ~~each~~ PCR product in each well

|     | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|
| 1Kb | Ladder | Blank  | X      | \      | □      | Δ      | ■      | ▲      |

Gel run at 100 V started at 5:57 PM

ended at 6:55 PM

Results:

- See teamspace folder
- All bands below .5 Kb

Location of product: N/A

Label on product: N/A

End Notes/Comments:

NEXT STEP:

Continued on back? Yes ☐; No ☒

Date and Time: 1-31-13Lab Technicians(s) involved: Matt Mortenson  
Andra GarveyProtocol: Growth Liquid CultureReagents: Chloramphenicol  
chloro

Procedure (with applicable notes):

~~5 mL LB~~  
 • 4999 ~~μL~~ 1 μL LB } 10 μg/mL  
 • 1 μL chloro  
 • 1 control was made with BBA-215104 to test the chloro.

Results: Success!

TBD in the warm room  
 - Yes Z, BglS, and XynA grew  
 - K215004 was killed by the antibiotic (chloro)

Location of product: Warm RoomLabel on product: Yes Z biobrick, Xna biobrick, BglS biobrick, BBA-K215104

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Mini PrepContinued on back? Yes ☐; No ☒



Date and Time: 2/1/14

Lab Technicians(s) involved: MWH

Protocol: Miniprep

AG

Reagents: XynA, YesZ, +Bgls biobrick from p13

QIA Gen miniprep kit

## Procedure (with applicable notes):

5 mL of Liquid culture of XynA, YesZ, +Bgls Biobrick spun for 3 minutes @ 8000 rpm  
 supernatant removed, pellet resuspended in 250  $\mu$ L P1, lysed 250  $\mu$ L P2, neutralized w/ 250  $\mu$ L N3  
 10,000 rpm for 10 min spun. Supernatant put into spin column; spun  
 Washed w/ 500  $\mu$ L Buffer PB. spun  
 Washed w/ 750  $\mu$ L Buffer PE spun for 1 minute  
 Eluted w/ 50  $\mu$ L Buffer EB, ~~spun~~ (sit for 1 minute, spun for 1)  
 Product moved to microcentrifuge tubes.

## Results:

1 50  $\mu$ L microcentrifuge tube of DNA per gene

Qubit couldn't be done because standards are missing

Qubit done 2/3/14

Bgls: 600  $\mu$ g/mLXynA: 12.4  $\mu$ g/mLYesZ: 7.24  $\mu$ g/mL

Don't use these measurements

Bgls 8.49  $\mu$ g/mL

XynA 11.1

YesZ 5.31

Taken from uncalibrated Qubit

Location of product: -20° GEM plasmids Box

Label on product: &lt;Gene Name&gt; Biobrick 2/1/14 p14 AG Chloro

End Notes/Comments:

## NEXT STEP:

Determine concentration + PCR

Continued on back? Yes ☐; No ☐

Date and Time: 2-2-13  
 Protocol: PCR  
 Reagents: Q5 2X Master Mix  
BglS BB Primer Rev + For  
XynA BB Primer Rev + For  
YesZ BB Primer Rev + For

Lab Technicians(s) involved: Anna GARVEY  
MATT MORTENSON

Procedure (with applicable notes):

W.A 25mL solution was made

'all was done on ice

- 3 were ~~used~~ <sup>done</sup> with BioBrick Primer (BglS, XynA, YesZ)
- 3 were done with the primers from the primer sheets

Results:

DNA was replicated.

~~BB~~ <sup>BglS</sup> done 2/3/13

BglS: 44.4 ug/mL

XynA: 5.88 ug/mL

YesZ: 4.89 ug/mL

Location of product: PCR Product Box

Label on product: BglS BB, XynA BB, YesZ BB

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Run on gel

Continued on back? Yes ☐; No ☐

Date and Time: 11 pm 2-1-14  
Protocol: Liquid Culture  
Reagents: B0034 pSB1A3  
Ampicillin

Lab Technicians(s) involved: AG  
MWM

## Procedure (with applicable notes):

Made 4 5mL Liquid cultures w/ Ampicillin concentration of 100ug/mL  
Cultured w/ B0034 on pSB1A3 from liquid culture  
left in warm room

## Results:

TBD

Growth

Location of product: Warm Room

Label on product: B0034 pSB1A3 MWM 2-1-14

End Notes/Comments:

## NEXT STEP:

Miniprep

Continued on back? Yes ☐; No ☐

Date and Time: 2-2-14  
Protocol: Miniprep  
Reagents: B0034 Liquid Culture  
Spin Miniprep kit (QIAGEN)

Lab Technicians(s) involved: MWM

Procedure (with applicable notes):

Continued from p16 - B0034 liquid culture spun down/pelleted  
Miniprep run, consolidating all the LCs into 1 spin column.  
Qubit analysis to determine concentration

Results:

1 50 $\mu$ L elution of B0034 Plasmid @ 58.9 $\mu$ g/mL

Location of product: GEM Plasmid Box  
Label on product: B0034 PSB1A3 MWM P16 2-2-14  
End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 2-2-14  
 Protocol: Electrophoresis  
 Reagents: Gel Green in DMSO  
~~loading~~

Lab Technicians(s) involved: AG  
 \_\_\_\_\_  
 \_\_\_\_\_

Procedure (with applicable notes):

A gel was made w/ .625g of agarose in 50mL TAE IX Buffer, + 2.5µg of Gel Green.

|        |   |      |      |      |      |      |      |
|--------|---|------|------|------|------|------|------|
| 1      | 2 | 3    | 4    | 5    | 6    | 7    | 8    |
| Ladder |   | BglI | XbaI | HesZ | BglI | XbaI | HesZ |
|        |   | BB   | BB   | BB   |      |      |      |

Results:

The results were unfavorable. \*See gel picture

Location of product: N/A

Label on product: N/A

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Find problem / Troubleshoot → Mini prep failure  
 → PCR failure

Continued on back? Yes ☐; No ☐



Date and Time: 5:00 2/3/14  
 Protocol: Gel  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: Daniel Schneider  
Anna - took gel off

Procedure (with applicable notes):

- ps. 15 (BioBricks/PCR products)
- ps. 14 (Mnirp products)
- ps. 15 (yes Z; alternative primer)
- 1% gel; 5 ml DNA; 1 ml loading dye

|    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|
| L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 |
| □  | □  | □  | □  | □  | □  | □  | □  |

L1 = ladder

L2 = ~~mp B~~

L3 = ~~mp~~ yes Z

L4 = ~~mp~~ X

L5 = PCR B(~~BB~~)

L6 = PCR X(BB)

L7 = PCR Y(BB)

L8 = PCR Y

messed up →

Results:

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:



Date and Time: 2-4-14 9:35pm  
Protocol: Quadrant Plating  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: AG  
MM  
\_\_\_\_\_

Procedure (with applicable notes):

- Quadrant streaking w/ just LB plates
- no antibiotic in plates
- 3 plates

Results:

TBD

Location of product: Warm Room

Label on product: B. subtilis 168

End Notes/Comments: \_\_\_\_\_  
\_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 2/4/14 19m  
Protocol: Liquid Culture  
Reagents: B. sub 168

Lab Technicians(s) involved: MWM  
AG

## Procedure (with applicable notes):

Made 9 5mL Liquid cultures (no antibiotic) of B. sub. 168

## Results:

Location of product: Warm Room

Label on product: B sub 168 2/4/14 MWM or AG

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Genome Isolation

Continued on back? Yes ☐; No ☐

Date and Time: 5 pm 2/11/14  
Protocol: Genome Isolation  
Reagents: QAT Gen Blood + Tissue Kit

Lab Technicians(s) involved: JR  
AG  
MM  
SVA

## Procedure (with applicable notes):

12 1.5 mL aliquots of B sub 168 culture  
Tubes battered for 5 hours, vortexed every 30 min

## Results:

4 200  $\mu$ L elutions 2 primary  
2 secondary

Location of product: Plasmid Box -20

Label on product: 5 Sub 168 Genome Elution

End Notes/Comments: \_\_\_\_\_

NEXT STEP: PCR

Continued on back? Yes ☐; No ☐



## PCR

Date and Time: 2/5/14 5pmPerformed by: SV, GG, AG, AW

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer 2x master mix from Lambda BiotechdNTPs 2x master mixForward Primer BGLS # 95366342, XynA # 95366338, YesZ # 95366350Reverse Primer BGLS # 95366343, XynA # 95366339, YesZ # 95366351Template DNA Genome prep from pg23 ELUTION 1Taq DNA Polymerase 2x master mix vial 1Nuclease-free water mol bio H<sub>2</sub>O

## Procedure:

~~25 um master mix 1.5 ul~~  
~~1 um template pg23~~  
~~1.5 ul~~

O - everything

X - no primers

☐ - no template

Z - yes Z

A - xynA

S - bglS

| Symbol on lid | Content in PCR tube |
|---------------|---------------------|
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |

## Temperature Settings

|              |  |
|--------------|--|
| Denaturation |  |
| Annealing    |  |
| Extension    |  |
| Final        |  |

Number of cycles:  Time of completion:  Label on product(s):  Location of product(s):  

End notes/comments:

Not complete, tubes labeled - Drawer tube rack drawer & Haulkel carbon its - 2/5/14

## NEXT STEP:

Continued on back? Yes ☒; No ☐

OZ - 10  $\mu$ m mastermix 1  
1  $\mu$ m template - yes Z  
1  $\mu$ m yes Z forward  
1  $\mu$ m yes Z Rev  
7  $\mu$ m H<sub>2</sub>O  
20

OA - 10  $\mu$ m mastermix 1  
1  $\mu$ m template - xynA  
1  $\mu$ m xynA Fwd  
1  $\mu$ m xynA Rev  
7  $\mu$ m H<sub>2</sub>O  
20

OS - 10  $\mu$ m mastermix 1  
1  $\mu$ m template - bgl S  
1  $\mu$ m bgl S Fwd  
1  $\mu$ m bgl S Rev  
7  $\mu$ m H<sub>2</sub>O  
20

X1 - 10  $\mu$ m mastermix 1  
X2 - 1  $\mu$ m template  
X3 - 9  $\mu$ m H<sub>2</sub>O

□Z - 10  $\mu$ m mastermix  
1  $\mu$ m yes Z Fwd - yes Z  
1  $\mu$ m yes Z Rev  
8  $\mu$ m H<sub>2</sub>O  
20

□A - 10  $\mu$ m mastermix  
1  $\mu$ m xynA Fwd -  
1  $\mu$ m xynA Rev  
8  $\mu$ m H<sub>2</sub>O  
20

□S - 10  $\mu$ m mastermix  
1  $\mu$ m bgl S Fwd  
1  $\mu$ m bgl S Rev  
8  $\mu$ m H<sub>2</sub>O  
20

## PCR

Date and Time: 2/6/14 7:30 pm Performed by: SS, SV

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer \_\_\_\_\_

dNTPs \_\_\_\_\_

Forward Primer \_\_\_\_\_

Reverse Primer \_\_\_\_\_

Template DNA \_\_\_\_\_

Taq DNA Polymerase \_\_\_\_\_

Nuclease-free water \_\_\_\_\_

Procedure:

see previous information  
on page 24

on x2 I was short on master mix I  
and I put whatever was left in the container into x2 ~ 5µm

| Symbol on lid | Content in PCR tube |
|---------------|---------------------|
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |

on back of page  
24

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 60°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 36Time of completion: 11:30 PM

Label on product(s): \_\_\_\_\_

Location of product(s) 4°C freezer

End notes/comments:

Run I GEM pool from the iGEM 2013 folder at 20 µL

NEXT STEP:

Run PCR product on gel

Continued on back? Yes ☐; No ☒

Date and Time: 2-5-14  
Protocol: Agar plates  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: AG  
\_\_\_\_\_  
\_\_\_\_\_

Procedure (with applicable notes):

500 mL DI water  
to  
20g LB agar



5 LB  
5 LB Amp  
5 LB Kan  
5 LB Chloro

Results:

Plates!

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_  
\_\_\_\_\_

NEXT STEP:

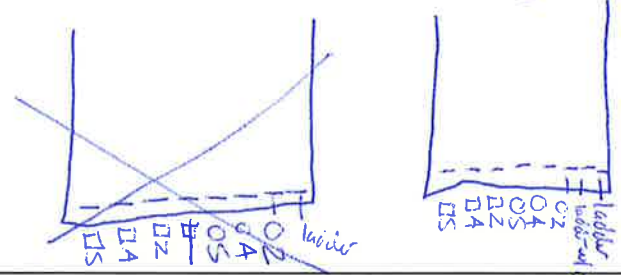
Continued on back? Yes ☐; No ☒

Date and Time: 2/7/14 6 pm  
 Protocol: Gel electrophoresis  
 Reagents: Agarose, TAE, GelGreen,  
 1 kb ladder, PCR products  
 O2, O4, O5, X1, X2, X3, D2, D4,  
 D5

Lab Technicians(s) involved: B

Procedure (with applicable notes):

Made 1% agarose gel  
 Found PCR products in 40C freezer  
 -made by GG on 2/5/14 (p24-25 in notebook)  
 Added 5  $\mu$ L PCR product and 1  $\mu$ L 6x loading dye to each lane  
 and 5  $\mu$ L 1 kb ladder to 1st lane. 5  $\mu$ L 1 kb ladder to lane 1 and 1.5  $\mu$ L 1 kb ladder w/ 0.3  $\mu$ L loading dye to lane 2



Run gel in TAE 1x buffer at 100V  
 Start: 7:10 pm  
 End: 8:05 pm

Results:

Inconclusive  $\rightarrow$  The ~~set~~ GelGreen used was is suspected to be ~~dry~~ old and has gone bad. The bands are incredibly faint even though the correct amounts of everything was added. A picture will be uploaded to Temspace just for kicks.

Location of product: 40C freezer

Label on product: GG 2/5/14  $\rightarrow$  O2, O4, O5, D2, D4, D5, X1, X2, X3

End Notes/Comments: Grrr

NEXT STEP:

Run PCR products X1, X2, X3, Rerun PCR products O2, O4, O5, D2, D4, D5

Make a

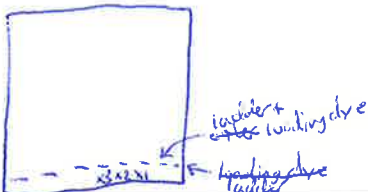
Continued on back? Yes ☐; No ☒

Date and Time: 2/7/14 7:30 PM  
 Protocol: Gel electrophoresis  
 Reagents: Agarose, TAE, Gel Green,  
Lab Ladder, PCR products,  
x1, x2, x3

Lab Technicians(s) involved: ZB  
JJ

Procedure (with applicable notes):

- First made 1% agarose gel, also prepared a second gel for next week  
 added the x1, x2, and x3 <sup>5 $\mu$ L</sup> from the <sup>4 $^{\circ}$ C</sup> freezer which were made 2/5/14,  
 and already had loading dye (matt said) in lanes 3, 4, 5  
 added 1.5  $\mu$ L lab ladder to lane one and 1.5  $\mu$ L lab ladder + 3  $\mu$ L loading dye to  
 lane two (cause we weren't sure which ladder worked better)  
 ran gel in TAE 1x buffer at 100v  
 start time: 8:54 pm



\* labeling system from page 24

Results:

Gel failed - ST

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 2/10/14 2:35pm

Lab Technicians(s) involved: ZB  
DS

Protocol: Gel electrophoresis

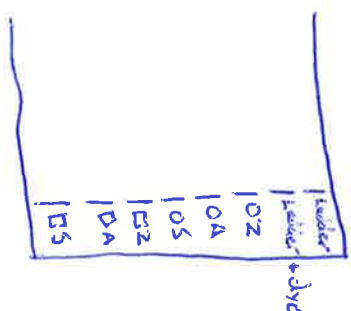
Reagents: Lab ladder, PCR products

02, 04, 05, 12, 14, 15

## Procedure (with applicable notes):

used Gel machine by myself on pg 28

- For a Ladder put 1.5ul in lane one, then put combine 1.5ul Lab ladder and 3ul loading dye, then in the rest of the lanes add the PCR products as shown below



Start 3:10 pm at 100v

0 = empty  
 □ = no template (control group)

was told by Dr. Hentle to, not do this

## Results:

→ only ladder showed

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐

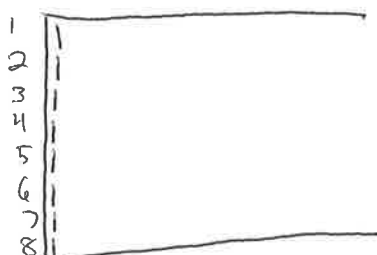
Date and Time: 2/10/14Lab Technicians(s) involved: R6Protocol: Gel electrophoresisReagents: 1x TAE Buffer, Agarose,GelGreen 10,000x (4°C stored)6x orange loading dye (BioLabs assembly),1kb DNA ladder (BioLabs assembly)

## Procedure (with applicable notes):

Made 1% agarose gel (50 mL TAE, .5g Agarose, 2.5 mL GelGreen)

★ Testing our 1kb ladder to see what amount is best to use ★

Att wa



1 1 mL  
2 1 mL w/ dye = .5 mL dye  
3 2.5 mL  
4 2.5 mL w/ dye = .5 mL dye  
5 5 mL  
6 5 mL w/ dye = 1 mL dye  
7 10 mL  
8 10 mL w/ dye = 2 mL dye

Ran gel at 100V  
Start: 5:55 pm

## Results:

The loading dye must be used w/ the 1kb ladder. The lanes w/ loading dye turned out clearly, the ones w/out did not. Picture will be uploaded to teamspace. 10 µL volume was most clear.

Location of product: N/ALabel on product: N/A

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

N/AContinued on back? Yes ☐; No ☒



## PCR

Date and Time: 2/10/2014 @ 5 pmPerformed by: Dan Schieder + Josiah Kieck + AC

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer 2x master mix from Lander BiotechdNTPs 2x master mixForward Primer BGLS #95366342; XynA #95366339; Yes Z #95366350Reverse Primer BGLS #95366343; XynA #95366339; Yes Z #95366351Template DNA Genome prep from p. 23 E. coliTaq DNA Polymerase 2x master mix vial 1Nuclease-free water mul by H<sub>2</sub>O

} Did the ones found in p. 20-23 in the 3gen info folder

## Procedure:

|  |  |  |   |   |
|--|--|--|---|---|
| 0 - everything<br>X - no primers<br>□ - no template                                    | Z = yes Z<br>A = XynA<br>S = BglS  | 0Z = 10µm master mix<br>3µm template (yes Z)<br>1µm yes Z forward<br>1µm yes Z Reverse<br>4µm H <sub>2</sub> O<br>20µm | 0A = 10µm master mix<br>3µm template (XynA)<br>1µm XynA forward<br>1µm XynA Reverse<br>4µm H <sub>2</sub> O<br>20µm | 0S = 10µm master mix<br>3µm template (BglS)<br>1µm BglS forward<br>1µm BglS Reverse<br>4µm H <sub>2</sub> O<br>20µm |
| 0Z = 10µm master mix<br>1µm yes Z fwd<br>1µm yes Z Rev<br>8µm H <sub>2</sub> O<br>20µm | 0A = 10µm master mix<br>1µm XynA fwd<br>1µm XynA Rev<br>8µm H <sub>2</sub> O<br>20µm | 0S = 10µm master mix<br>1µm BglS fwd<br>1µm BglS Rev<br>8µm H <sub>2</sub> O<br>20µm                                   | X1; 10µm master mix<br>3µm template<br>1µm H <sub>2</sub> O<br>20µm   |   |

| Symbol on lid | Content in PCR tube |
|---------------|---------------------|
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |
|               |                     |

## Temperature Settings

|              |  |
|--------------|--|
| Denaturation |  |
| Annealing    |  |
| Extension    |  |
| Final        |  |

16°C  
 16°C  
 16°C

Number of cycles: \_\_\_\_\_

Time of completion: \_\_\_\_\_

Label on product(s): see procedureLocation of product(s): -20°C freezer (with 16°C)

End notes/comments:

yes Z - 62°  
 BglS - 63°  
 XynA - 60.5° } denaturing temperatures  
 designed around the melting temperatures of the  
 primers

## NEXT STEP:

Run products on gel

Continued on back? Yes ☐; No ☐

Date and Time: 11:27pm 2/10/14  
Protocol: Liquid culture  
Reagents: LB Broth

Lab Technicians(s) involved: BT  
MM  
AG

## Procedure (with applicable notes):

K215010 - 30 this →  
K215004 - 30 this  
tops opened + taped

## Results:

Location of product: Worm room  
Label on product: K215010 - 1,2,3 K215004 - 1,2,3 / 2/10/14  
End Notes/Comments:

## NEXT STEP:

Mini prep

Continued on back? Yes ☐; No ☒

Date and Time: 2/11/14 9:00 am  
Protocol: mini Prep  
Reagents: K215 1010  
K214004

Lab Technicians(s) involved: AW, GG,  
MM

Procedure (with applicable notes):

mini prep kit procedure followed.

Products labeled:

AW 4 → From ~~K214004~~ K215104  
and ~~K215004~~  
AW 10 → From ~~K2151010~~  
K2150010

Results:

located in -20

Location of product: in microcentrifuge rack in -20, labeled w/ tape. Plasmid Box  
Label on product:  
End Notes/Comments:

NEXT STEP:

Try Double Transformation

Continued on back? Yes ☐; No ☒

Date and Time: 2/11/14 3:00pm  
 Protocol: Using 2 protocol not top 10  
 Reagents: DH5 Alpha cells

Lab Technicians(s) involved: MM  
GG  
SV

*Misc Box in -80°C  
 from "DH5α"*

Procedure (with applicable notes):

Followed online DH5 Alpha cell protocol  
 Heatshocked <sup>45 sec</sup> after 30 min on ice  
 added 1 mL LB Broth  
 put in warm room: 4:30 PM  
 removed at 5:30 PM 50 µL streaked on plates  
 1 mL put into 5 mL LB broth with 50 µg/mL Amp  
 placed on shaker plate at 27° in warm room at 6:30 PM  
 (200 rpm)

Results:

TBP next day

*2/12/14  
 cells used were not competent  
 They are also antibiotic resistant  
 Don't use for  
 further transformations*

Location of product: Warm room

Label on product: "10" transformation, "4" transformation, "4 + 10" (1) transformation, "4 + 10" (2) transformation

End Notes/Comments: Cells used were not labeled as competent

NEXT STEP:

If get cultures, pellet and observe fluorescence via UV light  
 In no cultures, redo with competent cells

Continued on back? Yes ☐; No ☒

Date and Time: 2/12/14Lab Technicians(s) involved: AG, RGProtocol: QubitReagents: Quant-it dsDNA BRAssay Kit, Miniprep products10 AW and 4 AW (from plasmid  
box in -20°C)

## Procedure (with applicable notes):

NOTE: Ratio of DNA sample will be 199:1

Initially put DNA solutions  
& standards in PCR tubes...  
transferred to Qubit tubesMade a working solution of 800  $\mu$ L

Made standards 1 &amp; 2

- 190  $\mu$ L working solution w/ 10  $\mu$ L standard

Made DNA solutions

- ~~190~~ 199  $\mu$ L working solution w/ 1  $\mu$ L 10 AW- 199  $\mu$ L working solution w/ 1  $\mu$ L 04 AW

## Results:

04 AW  $\rightarrow$  ~~197~~  $\mu$ g/mL 38.6  $\mu$ g/mL10 AW  $\rightarrow$  ~~157~~  $\mu$ g/mL 31.4  $\mu$ g/mLLocation of product: Plasmid Box -20° freezerLabel on product: 04 AW and 10 AW

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☒

Date and Time: 4:15pm, 2/12/14Lab Technicians(s) involved: ZBProtocol: plate streakingJSVReagents: K215010,K214004

## Procedure (with applicable notes):

I am testing whether the parts above are resistant to what ~~it~~ it says they are resistant to. ~~K215004~~ <sup>K21504</sup> should be resistant to CBoro and not to Amp or Kam, K215010 should be resistant to Amp, but not Chloro or Kam. I streaked each part onto an Amp, Kam, Chloro and LB plate to test all possible controls. Placed them into the warm room overnight

## Results:

should be done later, but wrong parts were done anyways

K215010 had colonies on only Amp and LB

K215004 had colonies on all the plates

resistant to all we have!

It is the DIS ~~Cells~~ <sup>Alpha</sup> cells... Not the part

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☒

Date and Time: 2/12/14 8:45 PMLab Technicians(s) involved: JJProtocol: glycerol stockMWReagents: chloramphenicolampicillin

## Procedure (with applicable notes):

3 tubes of 5 ml each of K21510 resistance A2  
3 tubes of 5 ml each of ~~K215004~~ resistance C2  
K215104

incubating in the stir plate  
They are in the warm room as of 9:30 PM

## Results:

TBD

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

miniprep

Continued on back? Yes ☐; No ☒

Date and Time: 2/13/14 5:00PMProtocol: Restriction Digest

Reagents: \_\_\_\_\_

~~XbaI~~ XbaI SpeILab Technicians(s) involved: JRAWMM

## Procedure (with applicable notes):

- We did a 50  $\mu$ L restriction digest, 3 replicants of it. ~~(pg 37)~~ referenced from pg 32.
- 26.31  $\mu$ L DNA (1  $\mu$ g) K215104 - plasmid that was cut
    - 5  $\mu$ L NEBuffer 2.0
    - 1  $\mu$ L of each enzyme (XbaI) (SpeI)
    - 16.7  $\mu$ L DEPC water
  - Placed in hot water bath @ 37°C for 1 hr (7:35) to (8:35)
- NOTE - no controls were done

## Results:

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐



Date and Time: 2/14/14 (FO-12)  
Protocol: Agar  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: Dwelschell  
\_\_\_\_\_  
\_\_\_\_\_

Procedure (with applicable notes):

1.) 500mL Agar → 500mL H<sub>2</sub>O + 20g Agar

2.) autoclave

3.) ~~Label~~ dishes  
table

4.) spread on dishes

\* Note: borrowed

1000mL ~~Galley~~  
Erythromycin  
flask from  
stock room

Results:

Location of product: \_\_\_\_\_  
Label on product: \_\_\_\_\_  
End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 2/14 12:50pm  
Protocol: streaking  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: ZB  
AW  
GG

## Procedure (with applicable notes):

Bba\_K21S104, A2 -Amp resistant (from block C1)  
streaked on all plates but we had no Amp plates  
so we did Kara, Chloa and LB on this part (sort of  
as practice). Again Amp still has to be done

Placed them in the Warm room

## Results:

Location of product: \_\_\_\_\_  
Label on product: \_\_\_\_\_  
End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Make Amp plates

Continued on back? Yes ☐; No ☒

Date and Time: 2/12/14 8:00pmLab Technicians(s) involved: JSProtocol: amp platesSV

Reagents: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## Procedure (with applicable notes):

1 made 20 plates with 1uL ampicillin per 1mL LB broth

## Results:

Location of product: The plates are solidifying on the counter by the white board

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Package plates in the morning

Continued on back? Yes ☐; No ☐

Date and Time: 2:00PM 2/23/14Lab Technicians(s) involved: Sierra TackettProtocol: Glycerol Stock

Reagents:

Good Vibrio Media100% glycerol40% glycerolProcedure (with applicable notes): All Kanamycin

Vibrio Harveyi in Good Vibrio Media (.75 mL)  
Good Vibrio Media (.375 mL) / 100% glycerol (.375 mL)  
↳ labeled

Rest = .75 mL 40% glycerol  
.75 mL liquid culture

- E. coli pBBR1 - MCS2

- E. coli pBBR2 - MCS2 - mCherry

- E. coli pBBR1 - MCS2 - gfp mut3<sup>+</sup>

Results:

Frozen in -80°C

Location of product:

Freezer Orphan Box (-80°C) (4 stocks)

Label on product:

name (above) Kana, ST 2-23-14

End Notes/Comments:

For Summer Camp

NEXT STEP:

Continued on back? Yes ☐; No ☒

Date and Time: 2-25-14 8:30-7:10 amLab Technicians(s) involved: Matt MortensenProtocol: Liquid CultureAnna CarveyReagents: Kanamycin, LB Broth  
BBa-K1175028 BBa-K1175009

## Procedure (with applicable notes):

Made 4 5mL ~~Liquid Culture~~ LB broth conical tubes at 35ug/mL Kanamycin for each type of bacteria.

Inoculated 4 tubes with BBa-K1175028 from Glycerol stock and 4 with BBa-K1175009 from Glycerol stock.

Left in warm room @ 37°C on a 200 rpm shaker plate.

## Results:

Location of product: Warm RoomLabel on product: BBa-K1175028/009 mwm/AL 2-25-14

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Plasmid IsolationContinued on back? Yes ☐; No ☒

Date and Time: 2-25-14 (7:45 pm)Lab Technicians(s) involved: ANNA GARVEY  
Matt MORTENSONProtocol: Mini PrepReagents: Resuspension Buffer (P1)P2, NB, PB, PE,  
Elution Buffer

## Procedure (with applicable notes):

The pellets were resuspended in 250  $\mu$ l Buffer P1 and transferred to a microcentrifuge tube. 250  $\mu$ l Buffer P2 was added. The tube was 4-6 times. Added 350  $\mu$ l of buffer NB + inverted 4-6 times. The tubes were inverted 4-6 times. The supernatant was added QIAprep spin column. The spin column was washed in 500  $\mu$ l Buffer PB and centrifuged for 30-60s and discard the flow-through. The tube was washed by adding 750  $\mu$ l Buffer PE. Centrifuge for 30-60s. The tube was let stand for 1min. Place the spin column in a 1.5ml microcentrifuge tube. The DNA was eluted with 50  $\mu$ l Buffer EB. Centrifuge for 1min.  
stand for 1min

## Results:

TBD

Location of product: -4 freezer in labLabel on product: K1175009 PSB1K3 AG 2-25-14 / K1175028 PSB1K3 AG 2-25-14

End Notes/Comments:

## NEXT STEP:

Qubit + Run Gel

Continued on back? Yes ☒; No ☐

K1175009

K1175028

→ these parts were miniprep'd from 4 different microcentrifuge tubes per part

## PCR

Date and Time: 2-28-14Performed by: Matt Mortensen Anna Garvey

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer NEB Q5 High Fidelity 2X master mix  
 dNTPs (master mix) 12.5 uL  
 Forward Primer FWD XynA (PCR Box) 1.25 uL  
 Reverse Primer Rev comp Rev XynA (PCR Box) 1.25 uL  
 Template DNA B Sub sp16 168 Genome Isolation product (23) 7.5 uL  
 Taq DNA Polymerase (master mix)  
 Nuclease-free water Dep C 2.5 uL

## Procedure:

4 tubes done w/ the above mix  
 Negative 1 without Primers +2.5 more water  
 Negative 2 w/o Template DNA +7.5 more water  
 Negative 3 w/o master mix +12.5 uL more water

| Symbol on lid | Content in PCR tube                 |
|---------------|-------------------------------------|
| X 1           | Mixture described above replicate 1 |
| X 2           | — rep 2                             |
| X 3           | — rep 3                             |
| X 4           | — rep 4                             |
| N 1           | Negative 1                          |
| N 2           | Negative 2                          |
| N 3           | Negative 3                          |

## Temperature Settings

|              |                   |
|--------------|-------------------|
| Denaturation | 95°               |
| Annealing    | <del>60</del> 62° |
| Extension    | 72°               |
| Final        | 72                |

Number of cycles: 34x2-28-14 AG p.44Time of completion: 8:30pmLabel on product(s): XynA PCR Product from GenomeLocation of product(s) -20 PCR Box

End notes/comments:

A gel is being done on the PCR Products

## NEXT STEP:

Run a Gel

Continued on back? Yes ☐; No ☐



Date and Time: 2-28-14  
 Protocol: Gel Electrophoresis  
 Reagents: Loading Dye 10X  
Gel Red  
Agarose  
TAE 1X Buffer

Lab Technicians(s) involved: ANNA GARVEY  
Matthew Mortensen

Procedure (with applicable notes):

5g of agarose was added to 50ml of TAE to cast gel

| Lanes  | 2 | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
|--------|---|----|----|----|----|----|----|----|
| Ladder |   | X1 | X2 | X3 | X4 | N1 | N2 | N3 |

replicate from PCR product done on XynA

negative controls

top-right hand side →

← top-left hand side

-gel Red had to be used for there was no gel Green available

Results:

The bands for the XynA gene were  $\approx 600 - 700$ bps. These results were negative for ~~XynA~~ XynA is 1179bps. The negative controls done show that the procedure was done correctly. This indicates a primer problem.

Location of product: N/A  
 Label on product: N/A  
 End Notes/Comments: Henkel was e-mailed about order new primers

NEXT STEP:

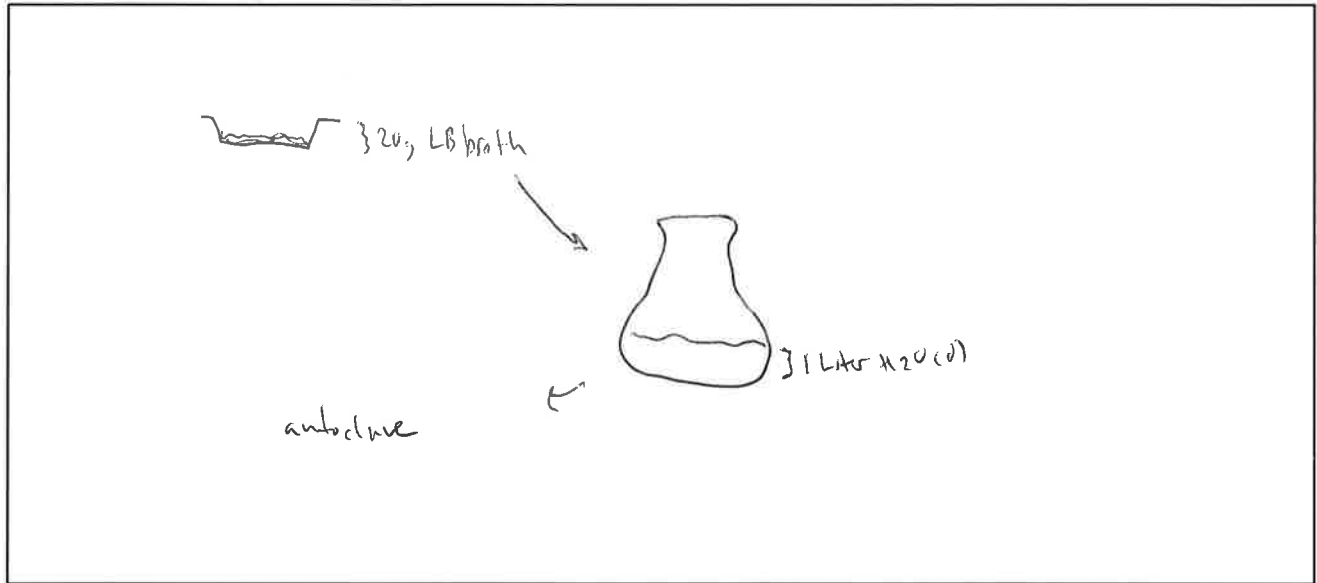
Reattempt with new primers.

Continued on back? Yes ☐; No ☐

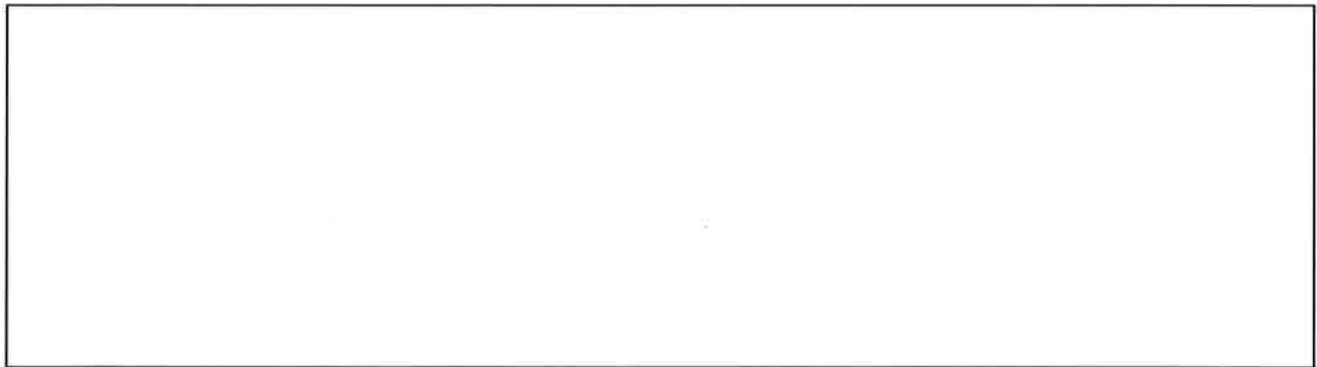
Date and Time: 8/4/17  
 Protocol: LB broth  
 Reagents: LB broth (20%)  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: Daniel Schwartz  
 \_\_\_\_\_  
 \_\_\_\_\_

Procedure (with applicable notes):



Results:



Location of product: on counter  
 Label on product: LB broth - secretion pump  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:



Continued on back? Yes ☐; No ☐

Date and Time: 3/4/14  
 Protocol: liquid culture amplification  
 Reagents: LB broth, ampicillin,  
resistance K215010,  
K215104  
chloramphenicol

Lab Technician(s) involved: Zach Biser

Procedure (with applicable notes):

K215010 and  
K215104.

create two liquid cultures for each part <sup>one with ~~ampicillin~~ chloro</sup>  
~~resistance~~ (control) and the other with amp. ~~resistance~~ add  
 Allow them to grow overnight in 37°C room  
 put in at 9:45pm

Results:

It was successful, both parts grew in amp. non grew in chloro

Location of product: warm room

Label on product: LB Amp<sup>chloro</sup> + part (K215104 or K215010)

End Notes/Comments: take down next step within 20 hrs

NEXT STEP:

Continued on back? Yes ☐; No ☒

## PCR

Date and Time: 3-4-14 9 pm

Performed by: Matt Mortensen

Reagents: (customize the list and include volumes and concentration):

|   |  |              |
|---|--|--------------|
| ThermoPol or Standard Taq Reaction Buffer | NEB Q5 High Fidelity 2x Master Mix           | 12.5 $\mu$ L |
| dNTPs                                     | MM   |              |
| Forward Primer                            | Fwd Bgl (PCR Box)                            | 1.25 $\mu$ L |
| Reverse Primer                            | Rev comp rev Bgl (PCR Box)                   | 1.25 $\mu$ L |
| Template DNA                              | B-sub sub 16S genome isolation product (p32) | 7.5 $\mu$ L  |
| Taq DNA Polymerase                        | MM   |              |
| Nuclease-free water                       | Dep C  | 2.5 $\mu$ L  |

## Procedure:

4 Tubes w/ mixture described above (25  $\mu$ L reactions)  
 Negative 1 no Primers + 2.5  $\mu$ L extra water  
 Negative 2 no Template DNA + 7.5  $\mu$ L extra water  
 Negative 3 no Master Mix + 12.5  $\mu$ L extra water

| Symbol on lid | Content in PCR tube                                    |
|---------------|--|
| B1            | "Mixture Described in Reagents section - replicate" #1 |
| B2            | " " " #2   |
| B3            | " " " #3   |
| B4            | " " " #4   |
| N1            | "Negative Control" #1 No Primers                       |
| N2            | " " #2 No Template DNA                                 |
| N3            | " " #3 No Master Mix                                   |

## Temperature Settings

|              |    |
|--------------|----|
| Denaturation | 95 |
| Annealing    | 63 |
| Extension    | 72 |
| Final        | 72 |

Number of cycles: 34

Time of completion: 9:30 am 4-5-13

Label on product(s): Bgl PCR Product/Negative

Location of product(s) PCR Box -20°C

End notes/comments:

Negatives can be ~~thrown~~ thrown out once a Gel is run

## NEXT STEP:

Run Gel

Continued on back? Yes ☐ ; No ☐

Date and Time: 3/5/14 5:00  
 Protocol: mini prep  
 Reagents: lots listed either  
below or on protocol sheets

Lab Technicians(s) involved: Daniel Schroeder  
Zach Birner

Procedure (with applicable notes):

Miniprep product from page 47, following procedure on pg 7 of iGEM protocols,

Then run a restriction digest following Lab Restriction Digestion protocol using DNA, dH<sub>2</sub>O, NEB Buffer 2, Xba I + Spe I. (No BSA, it's in NEB). Then place it in the thermal cycler (37°C 1hr, 65°C 20min) and then -20°C freezer for storage.

- also created liquid cultures for parts JO4450<sup>(36)</sup> (kan res) and K1175001 (kan res) (F2), and allowed them to grow over night in 37°C room

Results:

Location of product: liquid culture in warm room, miniprep/digest in iGEM plasmid box (-20°C freezer)  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 4-5-14 9pm  
Protocol: Liquid Culture  
Reagents: K1175028 (K), Kam,  
LB Broth

Lab Technicians(s) involved: Matthew Morlenton  
Anna Garvey

Procedure (with applicable notes):

35  $\mu$ g/mL Kanamycin x 3 K1175028 cultures (5 mL)

Results:

Location of product: Warm Room

Label on product: K1175028 Kam

End Notes/Comments: For Herkel (sequencing) after miniprep

NEXT STEP:

Miniprep

Continued on back? Yes ☐; No ☐

Date and Time: 3-5-14 10 pm  
Protocol: Gel Electrophoresis + Gel Extraction  
Reagents: PCR Product p48

Lab Technicians(s) involved: Matt Mortensen  
Anna Gargay

Procedure (with applicable notes): 1% Gel

| <u>L</u>   | <u>B1</u> | <u>B2</u> | <u>B3</u> | <u>B4</u> | <u>N1</u> | <u>N2</u> | <u>N3</u> |
|--|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <p>L = Ladder (1 kb)<br/>B1-4 = PCR Amplifies<br/>N1-3 = PCR Negatives (p48)</p> <p>5 Ladder + 1 Loading Dye<br/>10 <math>\mu</math>l Samples + 2 Loading Dye<br/>Used 2 <math>\times</math> 6 <math>\mu</math>l Gel green</p> |           |           |           |           |           |           |           |

Results:

|  |
|--|
|  |
|--|

Location of product: \_\_\_\_\_  
Label on product: \_\_\_\_\_  
End Notes/Comments: \_\_\_\_\_

NEXT STEP:

|  |
|--|
|  |
|--|

Continued on back? Yes ☐; No ☐

Date and Time: 3-5-14Lab Technicians(s) involved: ANNA GARVEYProtocol: Gel Electrophoresis + Gel Extraction for XynAMATTHEW MORTENSONReagents: Gel GreenGel Extraction Kit

## Procedure (with applicable notes):

Lanes

|        |                |                |                |   |   |   |   |
|--------|----------------|----------------|----------------|---|---|---|---|
| 1      | 2              | 3              | 4              | 5 | 6 | 7 | 8 |
| Ladder | X <sub>1</sub> | X <sub>2</sub> | X <sub>3</sub> | 5 | 4 | 7 | 8 |

- No negative controls were used for this gel was already done and XynA already confirmed

X<sub>1</sub>: 298.3 mg    X<sub>2</sub>: 209.6 mg    X<sub>3</sub>: 167.5 mg    X<sub>4</sub>: 235.1 mg

894  $\mu$ L    627  $\mu$ L    501  $\mu$ L    705  $\mu$ L

298    209    167    235

Q6  
Isopropanol

## Results:

Location of product: -20° pink rackLabel on product: XynA from Genome Purified PCR Product 3-5-14

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐



Date and Time: 8-6-14Lab Technicians(s) involved: Matt MarkisenProtocol: MiniprepReagents: Qiagen Spin Miniprep kitLiquor  
CulturesK1175028 (p53)JC4450K1175001

## Procedure (with applicable notes):

3 x K1175028 Emb cultures (MWM/AG)

2 x JC4450 and K1175001 (ZB/D.S.)

Protocol followed as written

Elution Buffer used

## Results:

3 50 uL Elutions

1 K1175028

1 JC4450

1 K1175001

Location of product: Plasmid Box -20°Label on product: Specimen 75B1K3 Plasmid 8-6-14 MWM p53

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

K1175028 Sequencing (Hershel)

JC4450 + K1175001 Res Dig

Continued on back? Yes ☐; No ☐

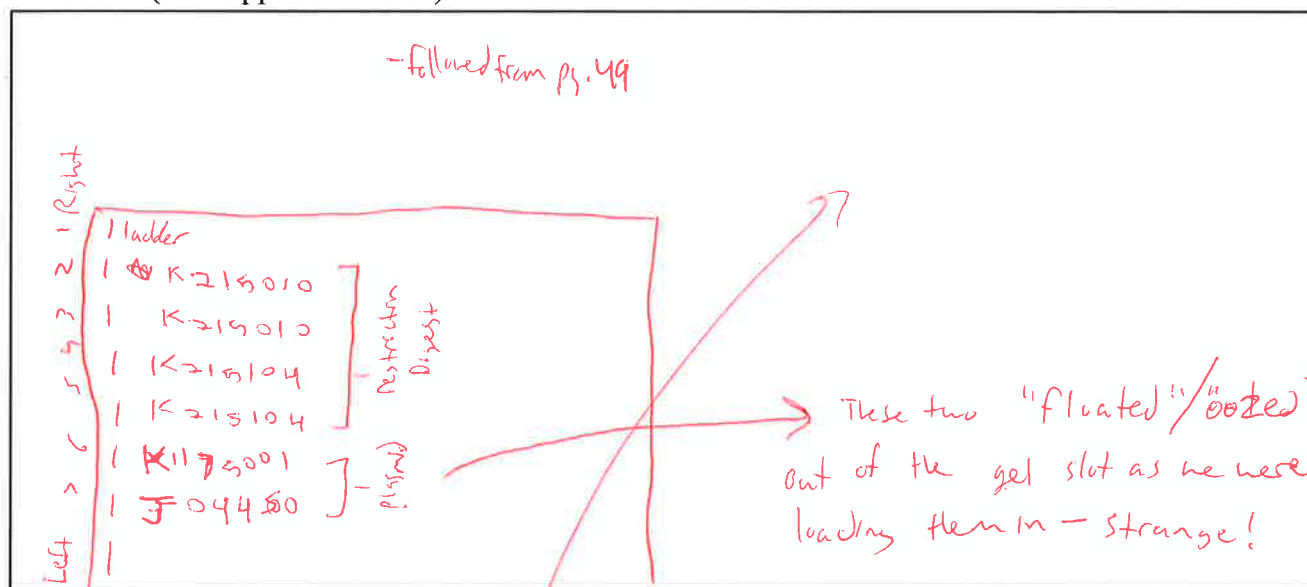
Date and Time: 3/14/14 5:00 pm

Protocol: Gel electrophoresis

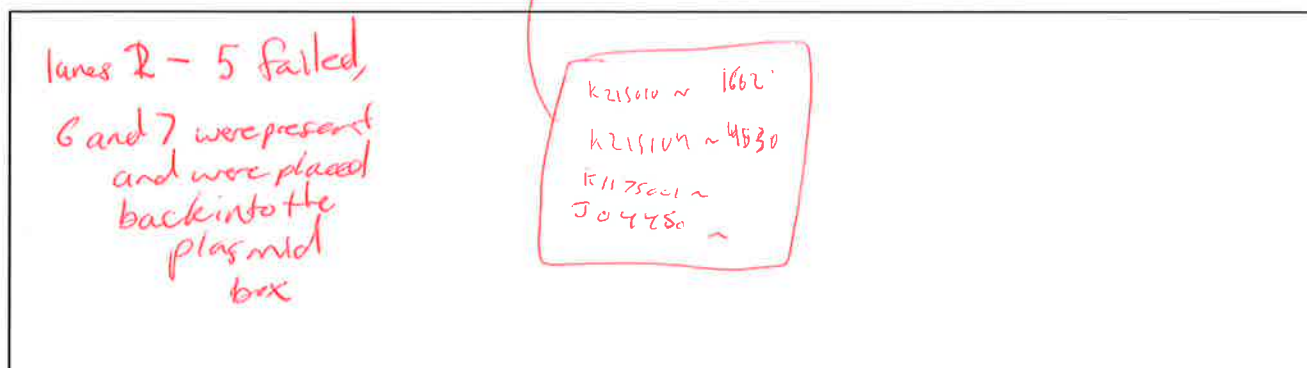
Reagents: parts: k215010,  
k215104, k1175001, J04400

Lab Technicians(s) involved: Daniel Schreder  
Zach Birner

Procedure (with applicable notes):



Results:



Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 3/19/14 7:30 PMLab Technicians(s) involved: Amel SchneiderProtocol: Coli electroporationReagents: parts: K215010; K215104

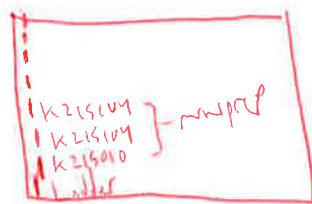
## Procedure (with applicable notes):

→ miniprep leftovers (we are testing this since the restriction digest failed on the previous page)

W

1 sample of K215010

2 samples of K215104



## Results:

showed up, picture online,  
threw away the gel

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 3/19/14 8:45pmLab Technicians(s) involved: Josiah Riche  
Ryan GeorgeProtocol: Liquid CultureReagents: LB brothAmpicillin KAChloramphenicol K(3)XynA (glycerol stock box 1-I)Pump (BBA-K215104) (glycerol stock box 1-A)

Procedure (with applicable notes):

Make 2 Liquid cultures

culture 1

5mL LB broth

10µL Amp

Pump bacteria

culture 2

5mL LB broth

1µL chlor

XynA

- Incubated at 37° overnight on shaker plate.

Results:

Location of product: Warm Room on shakerLabel on product: RG+JR pump and xynA

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Mini Prep tomorrow morningContinued on back? Yes ☒; No ☐math  
on  
back

$$C_1 V_1 = C_2 V_2$$

Amp

$$\left( \frac{50 \text{ mg}}{\text{mL}} \right) (V_1) = (100 \text{ mg/mL}) (5 \text{ mL})$$

$$V_1 = .01 \text{ mL} = 10 \text{ } \mu\text{L}$$

Chlor

$$\left( \frac{50,000 \text{ } \mu\text{g}}{\text{mL}} \right) (V_1) = (10 \text{ } \mu\text{g/mL}) (5 \text{ mL})$$

$$V_1 = .001 \text{ mL} = 1 \text{ } \mu\text{L}$$

Date and Time: 3/20/14 8:45amLab Technicians(s) involved: Ryan GeorgeProtocol: Min-prepReagents: Overnight culturesof XpA and pumpQiagen Spin min. prep kit

## Procedure (with applicable notes):

Centrifuged 1 mL of XpA and pump  
 Added to each tube: 250  $\mu$ L Buffer P1  
 Microcentrifuge 250  $\mu$ L Buffer P2 (set for 3 mins)  
 350  $\mu$ L Buffer N3

Centrifuged for 10 min at 13,000 RPM

Apply to spin column - centri: 30s, discard flow thru

Add .5 mL Buffer PB - centri: 30s, discard flow thru

Add .75 mL Buffer PE - " " " " "

Centri: 1min

Put each in a microcentrifuge tube - add 50  $\mu$ L Buffer EB, let stand 1min, centri: 1min

## Results:

Location of product: 40  $^{\circ}$ C fridge, pink rack

Label on product: RG min-peps pump & XpA

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Qubit DNA quantification

Continued on back? Yes ☐; No ☐

Date and Time: 3/20/2014 10:10 pmProtocol: liquid cultureReagents: LB, ampicillin,  
K215010, K215104Lab Technicians(s) involved: Zach Birner

## Procedure (with applicable notes):

\* created liquid cultures of the parts listed above in amp  
resistant LB broth

put them in warm room (37°C)  
at 10:40pm

## Results:

Location of product: warm room

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

mini prep / PCRContinued on back? Yes ☐; No ☐

Date and Time: 3/21/14 4:30pm  
 Protocol: WNI prep  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: David Sebach  
Zach Birner  
 \_\_\_\_\_  
 \_\_\_\_\_

Procedure (with applicable notes):

→ continued from p. 58

Results:

Location of product: Fridge (-4°C)  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐



PCR

Date and Time: 3/21/14 Performed by: Anna A and Josh Leverance

Reagents: (customize the list and include volumes and concentration): Carver

|   |   |                               |
|---|---|-------------------------------|
| ThermoPol or Standard Taq Reaction Buffer | <u>NEB Q5 High Fidelity 2x MasterMix</u>              | <u>12.5 <math>\mu</math>L</u> |
| dNTPs                                     | <u>MM</u>   | <u>-</u>                      |
| Forward Primer                            | <u>Fwd Bgls (PCR Box)</u>                             | <u>1.25 <math>\mu</math>L</u> |
| Reverse Primer                            | <u>Rev comp rev Bgls (PCR Box)</u>                    | <u>1.25 <math>\mu</math>L</u> |
| Template DNA                              | <u>B sub sub 168 genome isolation product (pg 32)</u> | <u>7.5 <math>\mu</math>L</u>  |
| Taq DNA Polymerase                        | <u>MM</u>   | <u>-</u>                      |
| Nuclease-free water                       | <u>Dep C</u>  | <u>2.5 <math>\mu</math>L</u>  |

Procedure:

4 Tubes w/ mixture described above (25  $\mu$ L reactions)

Negative 1 no Primers + 2.5  $\mu$ L extra water

Negative 2 no Template DNA + 7.5  $\mu$ L extra water

Negative 3 no MasterMix + 12.5  $\mu$ L extra water

| Symbol on lid | Content in PCR tube                                    |
|---------------|--|
| B1            | "Mixture described in Reagents section - replicate" #1 |
| B2            | " " #2   |
| B3            | " " #3   |
| B4            | " " #4   |
| N1            | "Negative Control" #1 No Primers                       |
| N2            | " " #2 Template DNA                                    |
| N3            | " " #3 No MasterMix                                    |

Temperature Settings

|              |    |
|--------------|----|
| Denaturation | 95 |
| Annealing    | 63 |
| Extension    | 72 |
| Final        | 72 |

Number of cycles: \_\_\_\_\_ Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_ Location of product(s) \_\_\_\_\_

End notes/comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐ ; No ☐

## Gel Electrophoresis

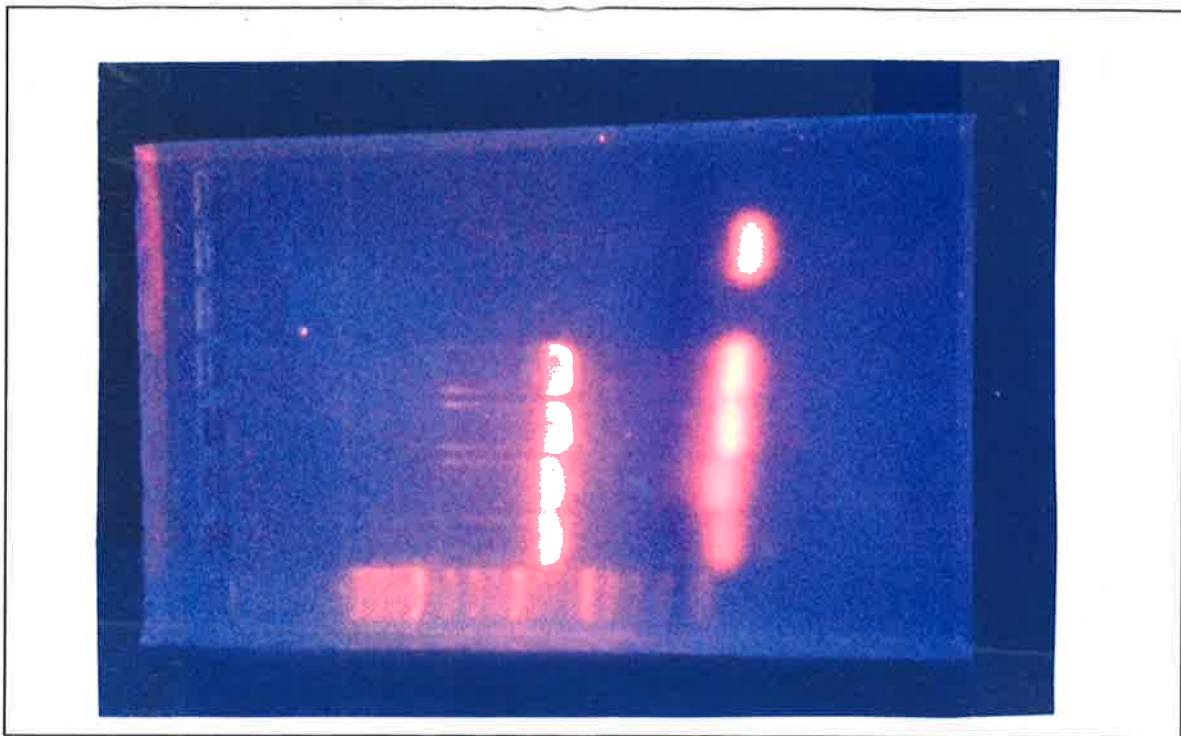
6)

JOSH LEVERANCE

Name: ANNA GARVEY

Date: \_\_\_\_\_

Time: \_\_\_\_\_



### Results

Gel electrophoresis showed that our PCR was successful. All four of our replicates showed a band size around 732 bp, which is the expected size of the *Bgl* gene.

NEXT STEP: ~~Get~~ Gel extraction

# Gel Electrophoresis

Page \_\_\_\_\_

Name: Anna, Matt, Josh

Date: 3/22/14

Time: 2:15 pm

% Agarose: 1.00

Lane Reagents

|   |                         |
|---|-------------------------|
| 1 | N3 - No<br>Master Mix   |
| 2 | N2 - No<br>Template DNA |
| 3 | N1 - No<br>Primers      |
| 4 | B4                      |
| 5 | B3                      |
| 6 | B2                      |
| 7 | B1                      |
| 8 | Ladder                  |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

- Loading dye (1xL)
- Gel Red
- Ladder - Quikload 2.0 kbp

Date and Time: 3-22-14 (4:10pm)  
 Protocol: Gel Extraction  
 Reagents: Quigen Gel Extraction Kit  
Isopropanol

Lab Technicians(s) involved: ANNA GARVEY  
JOSH LEVERANCE

Procedure (with applicable notes):

| Weights of tubes:           | B1    | B2    | B3    | B4    |
|-----------------------------|-------|-------|-------|-------|
| (g)                         | 1.062 | 1.100 | 1.080 | 1.056 |
| w/DNA gel                   | 1.267 | 1.294 | 1.317 | 1.337 |
| (mg)                        | 126.7 | 129.4 | 131.7 | 133.7 |
| Amount of buffer x3<br>used | 380.1 | 388.2 | 395.1 | 401.1 |

Results:

The ~~g~~ DNA was successfully extracted from the gel.

Location of product: PCR Box

Label on product: Bgls purified PCR #1

End Notes/Comments:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 3/22/14 4:26pm Performed by: Ryan George

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 2
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 900 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 4.5
4. Subtract #3 from #2 845.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #2 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

$$p_{ump} = 42.7 \mu\text{g/mL}$$

$$x_{pA} = 4.45 \mu\text{g/mL}$$

## Results

[illegible]

Date and Time: 3/22/14 5:15 pm Performed by: Ryan George

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer <sup>buffer 2.1</sup> NEB ~~cutsmart~~ → biobrick box, -20°C
- o DNA XynA and pump min-preps (pg. 57)
- o Enzyme SpeI, XbaI → Bio 202 & 360 enzymes box  
Freezer in Phillips lab

| MATERIALS | TYPE  | AMOUNT USED          |
|-----------|---|----------------------|
| Buffer    | <sup>buffer 2.1</sup> NEB <del>cutsmart</del> | 5 $\mu$ L            |
| DNA       | XynA  | 43 $\mu$ L           |
| Enzyme(s) | SpeI, XbaI                                    | 1 $\mu$ L, 1 $\mu$ L |
| Water     | DEPC  | 0                    |

Total Reaction Volume 50  $\mu$ L  
→ should have been 106  $\mu$ L if possible

Construct a table with this format for each reaction that you do today.

| Materials | TYPE  | Amnt used            |
|-----------|---|----------------------|
| Buffer    | <sup>buffer 2.1</sup> NEB <del>cutsmart</del> | 5 $\mu$ L            |
| DNA       | pump  | 23.4 $\mu$ L         |
| Enzyme(s) | SpeI, XbaI                                    | 1 $\mu$ L, 1 $\mu$ L |
| Water     | DEPC  | 19.6 $\mu$ L         |

TRV 50  $\mu$ L

Start Time of Incubation: 2: 6:25pm

End Time of Incubation: 6:35pm → can in thermal cycler

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37 °C

Method used for Quenching the Reaction (*different for different enzymes*)

Heat for 20 min at 65 °C

Where did you store your finished product and what did you label it?

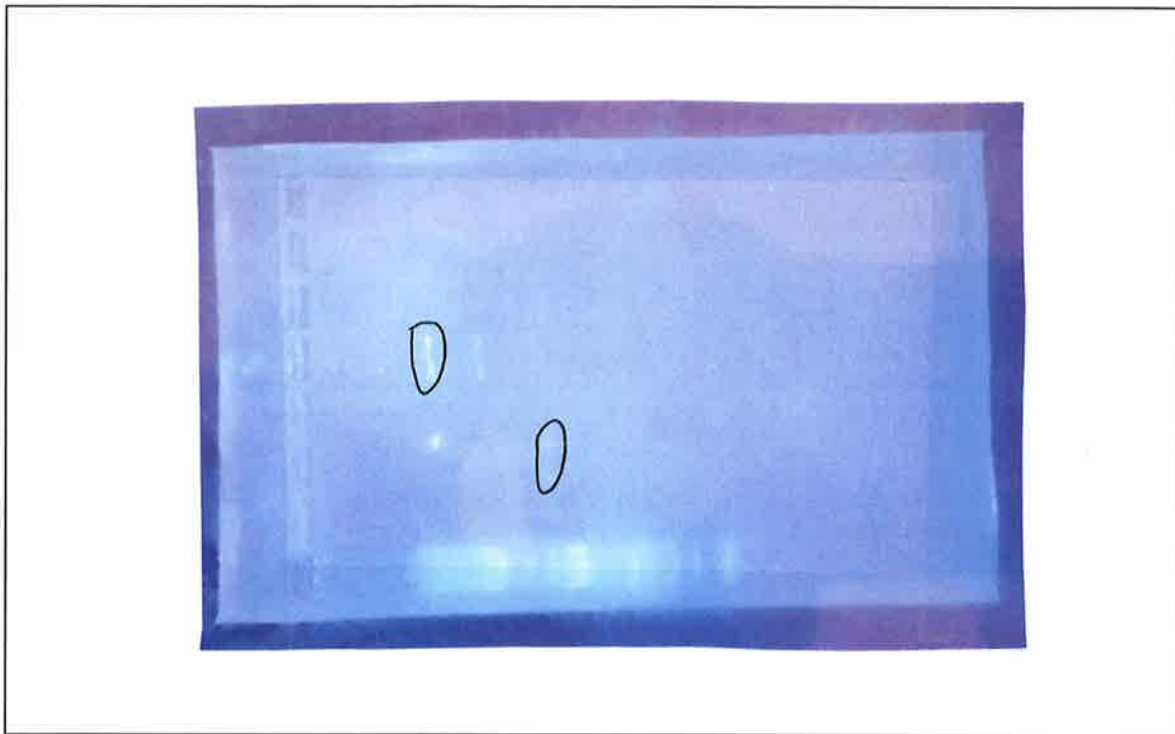
-20 °C freezer → Pump, XynA  
↓  
green tube rack      RG      RG

**NEXT STEP:**

1. Run a gel on the digests
2. Perform ligation of the two



## Gel Electrophoresis

Name: Ryan GeorgeDate: 3/23/14Time: 7:05 pm

## Results

Both xynA and the pump were correctly digested. The xynA has a band between 1 & 1.2 kb which is what we want (1179 bp for xynA). The pump is 4511 bp. There is a band b/w 4 & 5 kbp, which is what we want. Success!

NEXT STEP: Ligation

# Gel Electrophoresis

Page 64  
 Name: Ryan George  
 Date: 3/23/14  
 Time: 7:05 pm

% Agarose: 1

Lane Reagents

|   |        |  |
|---|--------|--|
| 1 | th     |  |
| 2 |        |  |
| 3 |        |  |
| 4 | pump   |  |
| 5 |        |  |
| 6 | x77A   |  |
| 7 |        |  |
| 8 | ladder |  |

Reminders:  
 Add Gel Green  
 Add Loading Dye  
 Run Red

Reagents used:

.5 g Agarose  
 50 mL 1x TAE  
 2.5 mL GelGreen

6 mL ~~th~~ ladder (Qwi:stent)

5 mL x77A }  
 5 mL pump } page 63

1 mL 6x Loading dye  
 ↑ for each sample

Start: ~~8:54~~  
 7:54 pm Voltage: 100V  
 End.

Name(s): Christa WinslowDate and Time: 3/25/14  
6:45pmLiquid CultureAmount of LB used: 5 mLPart Used: BBa\_K314200 (toxin gene)Antibiotic Used: chloramphenicolAmount of Antibiotic Used: 3  $\mu$ lConcentration of Antibiotic: 30  $\frac{\mu\text{g}}{\text{mL}}$ 

• used sterile "stick" instead of inoculating loop

did not grow; will need to be re-cultured on a later date 3/26/14 CW

Label on Product: BBa\_K314200 3/25 CWLocation of Product: warm roomNext Step: remove from warm room and perform mini prep

Name(s): Jacob SadatDate and Time: 25/3/14 7:40 PMLiquid CultureAmount of LB used: 5 mLPart Used: K875001Antibiotic Used: ChloramphenicolAmount of Antibiotic Used: 3  $\mu$ LConcentration of Antibiotic: 30  $\mu$ L/mLLabel on Product: K875001 cam IgemLocation of Product: warm room on shaker plateNext Step: miniprep

Name(s): Ryan George  
Josiah RickEDate and Time: 3/26/14 12:30Liquid CultureAmount of LB used: 5 mLPart Used: BBa\_K215001Antibiotic Used: C3 (Chloramphenicol)Amount of Antibiotic Used: 1 mLConcentration of Antibiotic: 10 µg/mLLabel on Product: K215001 C3 RLocation of Product: Warm Room shakerNext Step: Mini Prep

Date and Time: 3/26/14 5:20 PM Performed by: Zach Birner, Dan Schroeder

### Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

### PROCEDURE

#### Material Location

- Buffer NEB buffer 2.1
- DNA K215010, K215104
- Enzyme Spe I, Xba I

| MATERIALS | TYPE    | AMOUNT USED |
|-----------|---------|-------------|
| Buffer    | NEB 2.1 | 2.5 ml      |
| DNA       | K215010 | .5 ml       |
| Enzyme(s) | Spe I   | .5 ml       |
|           | Xba I   | .5 ml       |
| Water     | DEPC    | 21 ml       |

Total Reaction Volume 25 ml

Construct a table with this format for each reaction that you do today.

| Material | Type    | Amount |
|----------|---------|--------|
| Buffer   | NEB 2.1 | 2.5 ml |
| DNA      | K215104 | .5 ml  |
| Enzyme   | Spe I   | .5 ml  |
|          | Xba I   | .5 ml  |
| Water    | DEPC    | 21 ml  |

Total Volume: 25 ml

Start Time of Incubation: <sup>30<sup>sec</sup></sup>  
6:17 → <sup>got to 37°</sup> at 6:30 PM  
End Time of Incubation: ~~7:07~~ ~~7:42 PM~~ 6:52 PM

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): ~~37°~~ → 80°

Method used for Quenching the Reaction (different for different enzymes)

heat to stop it

30 minutes, 20 minutes

6:52 PM - 7:12 PM

Where did you store your finished product and what did you label it?

I ~~QEM~~ plasmid Box -20°C freezer, the part (k215010 or k215104) + res Dig. + initials

\* Note: I made a slightly bad mistake of putting them in at 30° (while was still heating up to 37°),  
so we are giving it an extra 5 minutes because of this

NEXT STEP:

<sup>to check</sup> Gel, ligation

## PCR

Matt Morkensen

Date and Time: 3/26/14 4:45 PMPerformed by: Anna <sup>^</sup> and Josh LeveranceReagents: (customize the list and include volumes and concentration): caney

ThermoPol or Standard Taq Reaction Buffer NEB Q5 High Fidelity 2x MasterMix 12.5  $\mu$ L  
 dNTPs MM -  
 Forward Primer yes-2 forward primer 1.25  $\mu$ L  
 Reverse Primer yes-2 reverse primer 1.25  $\mu$ L  
 Template DNA B sub sub 168 genome isolation product (pg 32) 7.5  $\mu$ L  
 Taq DNA Polymerase MM -  
 Nuclease-free water Pep D DEPC 2.5  $\mu$ L

## Procedure:

4 Tubes w/ mixture described above (25  $\mu$ L reactions)  
 Negative 1 no Primers + 2.5  $\mu$ L extra water  
 Negative 2 no Template DNA + 7.5  $\mu$ L extra water  
 Negative 3 no Master Mix + 12.5  $\mu$ L extra water

| Symbol on lid | Content in PCR tube                                    |
|---------------|--|
| Y1            | "Mixture described in Reagents section - replicate" #1 |
| Y2            | " " #2   |
| Y3            | " " #3   |
| Y4            | " " #4   |
| N1            | "Negative Control" #1 No Primers                       |
| N2            | " " #2 Template DNA                                    |
| N3            | " " #3 No Master Mix                                   |

## Temperature Settings

|              |    |
|--------------|----|
| Denaturation | 95 |
| Annealing    | 62 |
| Extension    | 72 |
| Final        |    |

Number of cycles: \_\_\_\_\_

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) \_\_\_\_\_

End notes/comments:

## NEXT STEP:

Continued on back? Yes ☐; No ☐



## Mini Prep

Name(s): Jacob Sedert, Christa Winslow  
 Date and Time: 7:30 PM 3/26/14

Check off as you  
complete the steps

**Procedure:**

Bacteria used: K875001

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1..... ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: K875001

Location of product: Plasmid box in  $-20^{\circ}\text{C}$

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Gel

Continued on back? Yes ☐; No ☒

# Gel Electrophoresis

Page 71

Name: Matt Mortensen Anne Garvey

Date: 5/26/14

Time: \_\_\_\_\_

% Agarose: 1

Lane Reagents

|   |    |  |
|---|----|--|
| 1 | Y1 |  |
| 2 | Y2 |  |
| 3 | Y3 |  |
| 4 | Y4 |  |
| 5 | N1 |  |
| 6 | N2 |  |
| 7 | N3 |  |
| 8 | L  |  |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used: B. Sub 169 Genome extracted YesZ (p69) 15uL + 3 Loading Dye (4 replicates) + 3 Negatives  
YesZ PCR Box P

1uL 1kb ladder + 5uL Loading Dye

5uL of Gel Red in a 1% Agarose Gel

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_



Picture

## Results

successfully isolated YesZ (1490 bp) - extracted.  
negatives as expected.

NEXT STEP:

Date and Time: 8/26/14 Performed by: Matt Mortensen Anna Garvda

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | Y1    | Y2    | Y3    | Y4    |  |  |  |  |  |
|-------------------|-------|-------|-------|-------|--|--|--|--|--|
| Tube + Gel weight | 1.18g | 1.19g | 1.20g | 1.25g |  |  |  |  |  |
| Empty tube weight | .948g | .934g | .918g | .921  |  |  |  |  |  |
| Gel Weight        | .232g | .256g | .282g | .329g |  |  |  |  |  |
| Buffer QG         | .696g | .768g | .846g | .987g |  |  |  |  |  |
| Isopropanol       | .232g | .256g | .282g | .329g |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100μL per 100mg; so 3 volumes is 300μL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800μL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750μL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30μL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: Yes-2 Bacillus subtilis 168

Location of Product:

Next Step:

## Mini Prep

Name(s): Ryan GeorgeDate and Time: 3/27/14 3:20 pmCheck off as you  
complete the steps**Procedure:**Bacteria used: BBA\_K215001

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1..... ☒  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: K215001 Min prep RGLocation of product:  $-20^{\circ}\text{C}$ 

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Qubit & restriction digestContinued on back? Yes ☐; No ☒

Date and Time: 3/27/14 4:20pm Performed by: Ryan George

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed 1
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 700 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 3.5
4. Subtract #3 from #2 696.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 3/27/14 4:50pm Performed by: Ryan George

### Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. **SO BE CAREFUL IN YOUR MEASUREMENTS**
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

### PROCEDURE

#### Material Location

- o Buffer biobrick assembly, -20°C
- o DNA tag m:prep (pg 73)
- o Enzyme Bio 202 & 360 enzyme box - molecular freezer

| MATERIALS | TYPE           | AMOUNT USED          |
|-----------|----------------|----------------------|
| Buffer    | NEB Bst 2.1    | 5 $\mu$ L            |
| DNA       | tag<br>K215001 | 43 $\mu$ L           |
| Enzyme(s) | SpeI, XbaI     | 1 $\mu$ L, 1 $\mu$ L |
| Water     | DEPC           | 0                    |

Total Reaction Volume 50  $\mu$ L  
 optimally 240.4  $\mu$ L

Construct a table with this format for each reaction that you do today.

1  $\mu$ g DNA

$$1 \mu\text{g DNA} \cdot \frac{1 \text{ mL}}{4.16 \mu\text{g}} \cdot \frac{1000 \mu\text{L}}{1 \text{ mL}} = 240.4 \mu\text{L}$$



Start Time of Incubation: 5:15 pm

End Time of Incubation: 6:15 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (different for different enzymes)

Heat @ 65°C for 20 mins

Where did you store your finished product and what did you label it?

-20°C freezer, tag RG

NEXT STEP:

Gel electrophoresis

-ligation w/ xNA & pump

# Gel Electrophoresis

Page 76

Name: Ryan George

Date: 3/27/14

Time: 6:35 pm

% Agarose: 1

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

|        |  |
|--------|--|
|        |  |
|        |  |
|        |  |
|        |  |
|        |  |
| tag    |  |
|        |  |
| ladder |  |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

2.5 mL Gel Red

50 mL 1x TAE

.5 g Agarose

Start: 6:49 pm @ 100 V  
end:

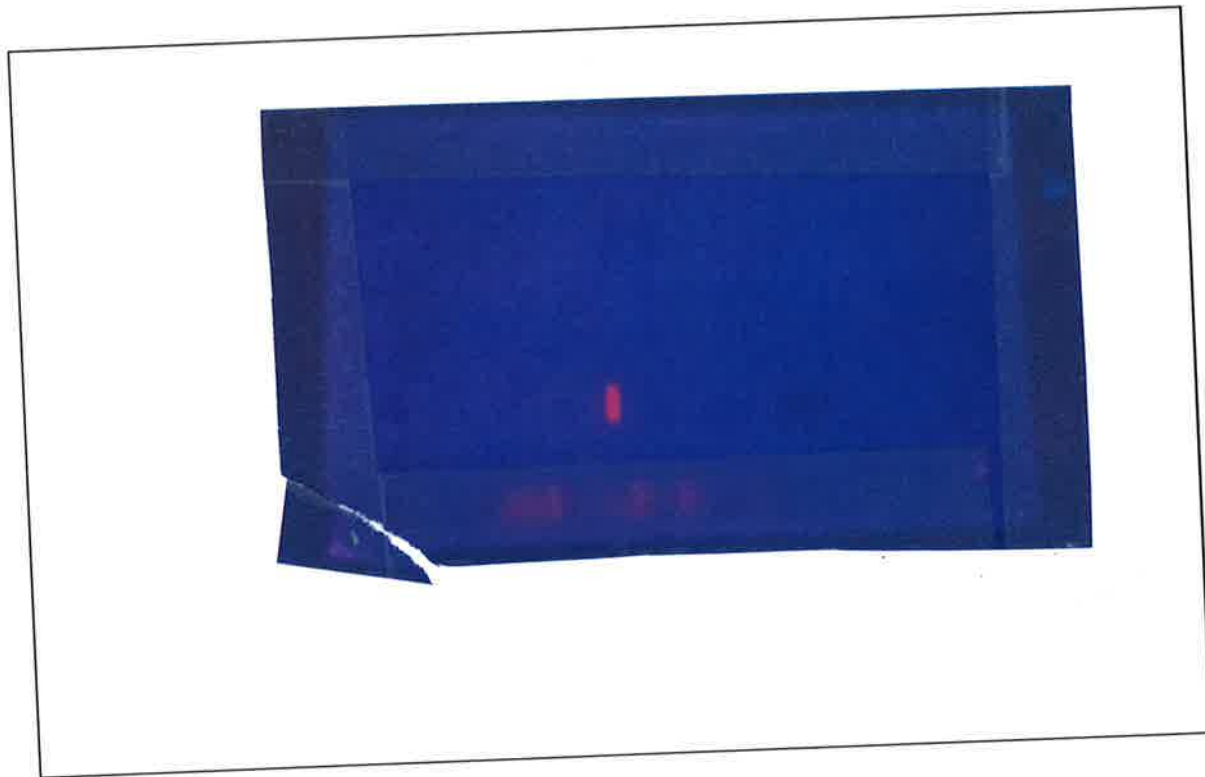
6  $\mu$ L <sup>2103</sup> ladder (quickstart)

5  $\mu$ L tag (BBA-K215001)

1  $\mu$ L 6x loading dye

## Gel Electrophoresis

Name: Ryan George  
Date: 3/27/14  
Time: 6:35 pm



Results

Digest  
Gel failed. Should be a band  $\sim 850$  bp (plus a second band for the rest of the plasmid). There was only 1 band @ 1.2 kb.

NEXT STEP:

Troubleshoot by running gel on mini-prep product for 4215001.

# Gel Electrophoresis

Name: Daniel Schmitt Page       
 Date: 3/27/14  
 Time: 2:45

% Agarose: 1

Lane Reagents

|   |                               |             |
|---|-------------------------------|-------------|
| 1 |                               |             |
| 2 | K25 (100%) Restriction digest | } secretion |
| 3 | K25 (100%) " "                |             |
| 4 | K25 (100%) " "                |             |
| 5 | K25 (100%) " "                | } pump      |
| 6 | K25 (100%) " "                |             |
| 7 | K25 (100%) " "                |             |
| 8 | ladder                        |             |

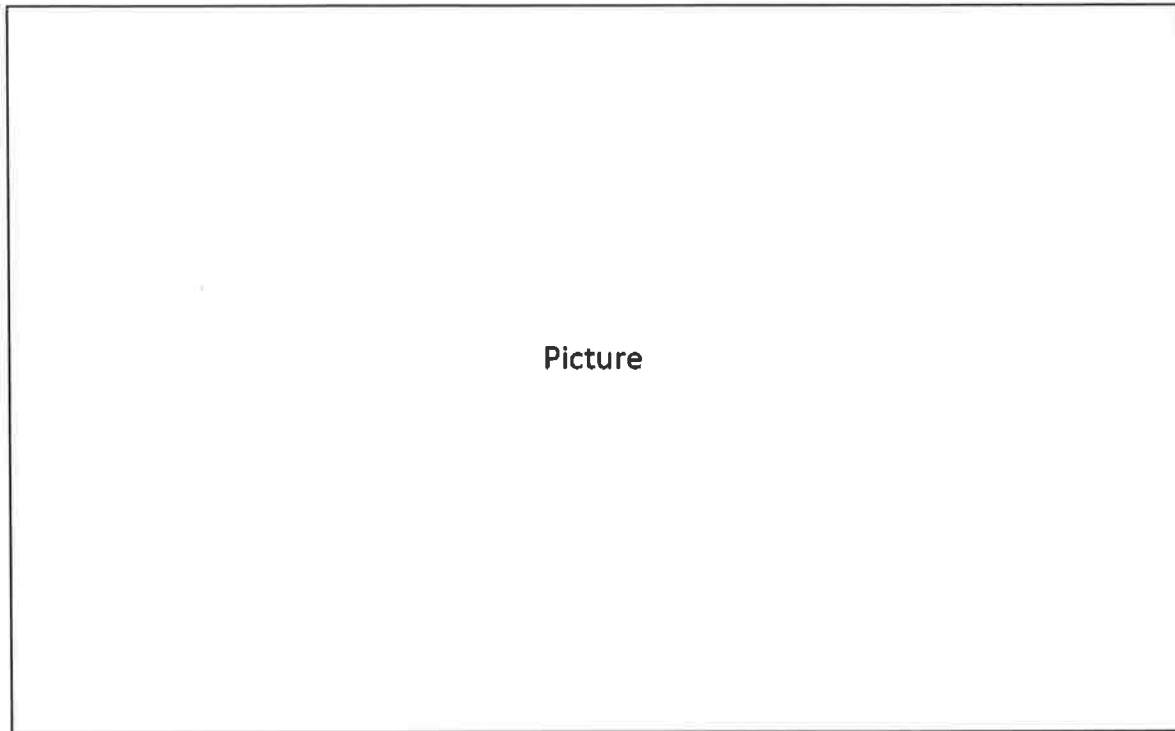
Reminders:  
 Add Gel Green  
 Add Loading Dye  
 Run Red

Reagents used:

→ 2.5ml Gel Red  
 50ml 1xTAE  
 1.5g Agarose  
 → 5ml 2x loading dye (amplified)  
 → 1ml 6x loading dye

# Gel Electrophoresis

Name: Daniel Schuch  
 Date: 3/28/14  
 Time: 1:50 2:45



Picture

## Results

|   |                  |
|---|------------------|
| 8 | K215010 R.D. (a) |
| 7 | K215010 R.D. (b) |
| 6 | K215104 R.D. (a) |
| 5 | K215104 R.D. (b) |
| 4 | 1004450 plasma   |
| 3 | K1175401 plasma  |
| 2 | Ladder           |

## NEXT STEP:

Run gel on plasma samples of K215104 & K215010

## Gel Electrophoresis

Page \_\_\_\_\_

Name: Anna Schaefer & Zarah Brunn

Date : 3/27/14

Time: \_\_\_\_\_

% Agarose: 1

| Lane | Reagents |
|------|----------|
|------|----------|

**1**

2

3

4

5

6

7

8

**Reminders:**

### Add Gel Green

### Add Loading Dye

## Run Red

$$h_2(5104)(a)$$
$$K_2 = 5104 (\text{B})$$

k.215010 (B)

$h_2 = 1500 \text{ V (A)}$

Widd

unipolar

**Reagents used:**

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_



Picture

Results

NEXT STEP:

# Gel Electrophoresis

Page \_\_\_\_\_  
 Name: Ryan George  
 Date: 3/30/14  
 Time: 5:45 pm

% Agarose: 1

Lane Reagents

|   |                   |   |
|---|-------------------|---|
| 1 | tag<br>mini-prep  | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | tag<br>mini-prep  |   |
| 3 | tag<br>digest     |   |
| 4 | ladder            |   |
| 5 | xvaA              |   |
| 6 | pump              |   |
| 7 |                   |   |
| 8 | <del>ladder</del> |   |

Reagents used:

Start: 6:17 pm @ 100v

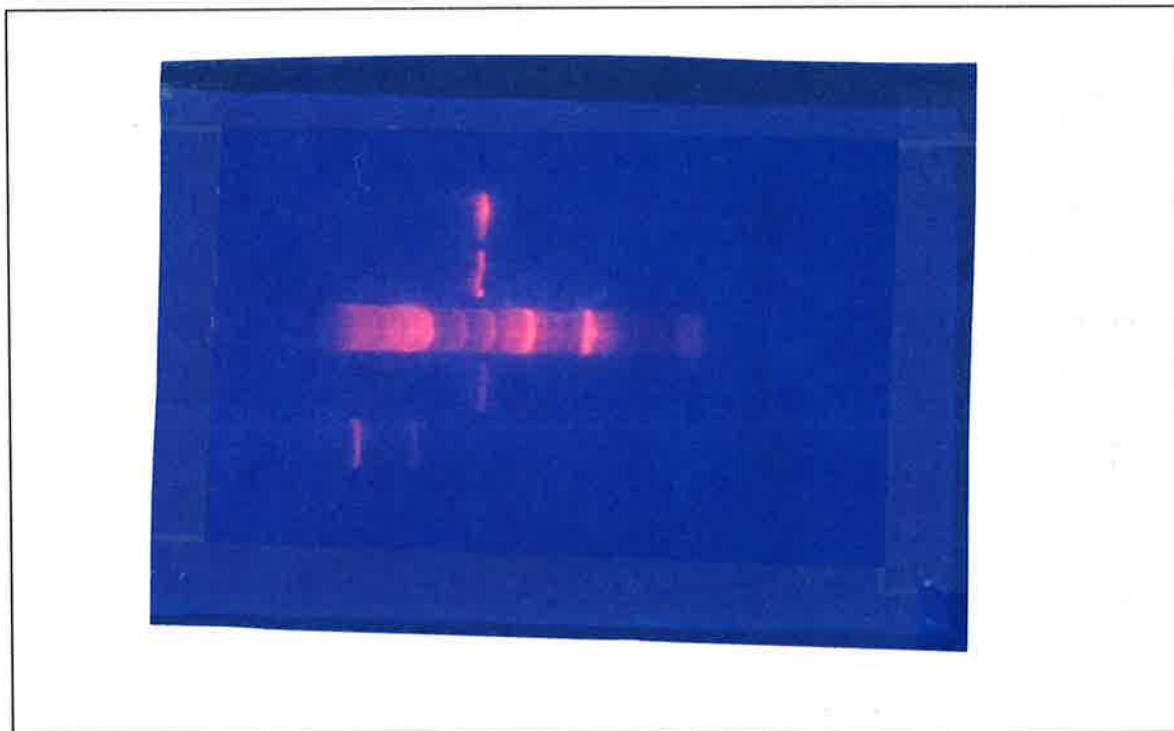
- 2.5 mL Gel Red
- 50 mL TAE
- .5g agarose

- 6 mL Quick-start 2 log ladder
- 1 mL loading dye (per sample)
- 5 mL of...
  - tag mini prep (pg. 73)
  - tag restriction digest (pg. 75)
  - xvaA digest } pg 63
  - pump digest }



## Gel Electrophoresis

Name: Ryan George  
Date: 3/30/14  
Time: 5:45 pm



## Results

- tag restriction digest did not work because the digest and the mini-prep have bands at the same spot ~ 1500 bp
- the pump is still good & usable
- xynA → found out there is an isolated & correct PCR product in -20°C all prior data on xynA for Ryan & Josiah was not needed.

NEXT STEP:

Name(s): Ryan GeorgeDate and Time: 3/30/14 6:20 pmLiquid Culture

|   |   |
|---|---|
| 1 | Amount of LB used: <u>5 mL</u>                              |
| 2 | Part Used: <u>BB<sub>4</sub> - K215001</u>                  |
| 3 | Antibiotic Used: <u>C3</u>                                  |
| 4 | Amount of Antibiotic Used: <u>1 <math>\mu</math>L</u>       |
| 5 | Concentration of Antibiotic: <u>10 <math>\mu</math>g/mL</u> |

|                      |                       |
|----------------------|-----------------------|
| Label on Product:    | <u>tag C3 R6</u>      |
| Location of Product: | <u>Shaw warm room</u> |
| Next Step:           | <u>min: - prep</u>    |

## Mini Prep

Name(s): JOSEPH RUCKE RYAN GEORGE  
 Date and Time: 3/31/14 7:30 pm

Check off as you  
complete the steps

**Procedure:**

Bacteria used: BBA - K215001

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1: kept in freezer..... ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: BBA-K215001 001 TAG MINI PREP JR  
 Location of product:  $-20^{\circ}\text{C}$

## Deviations from Procedure and other Notes:

NO DEVIATIONS WERE MADE TO THE PROCEDURE

## Successful?

## NEXT STEP:

Qubit and restriction digest

Continued on back? Yes ☐; No ☐

Date and Time: 3/31/14 8:15 pm Performed by: JOSIAH RICKS + RYAN GEORGE

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed extra
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 700 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 3.5  $\mu$ L
4. Subtract #3 from #2 696.5  $\mu$ L
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 9/1/14 11:30 am Performed by: JOSIAH RUCKE + RYAN GEORGE

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer Abm biobrick assembly - 20°C
- o DNA BDA-K215001 TAG from Mini Prep (81)
- o Enzyme ~~Xba~~ Xba I Spe I

| MATERIALS | TYPE        | AMOUNT USED         |
|-----------|-------------|---------------------|
| Buffer    | NEB 2.1     | 5 $\mu$ L           |
| DNA       | K215001 TAG | 10.42 $\mu$ L       |
| Enzyme(s) | Spe I Xba I | 1 $\mu$ L 1 $\mu$ L |
| Water     | DEPC        | 32.58 $\mu$ L       |

Total Reaction Volume 50  $\mu$ L

Construct a table with this format for each reaction that you do today.

$$1 \mu\text{g DNA} \cdot \frac{1 \mu\text{L}}{95.9 \mu\text{g}} \cdot \frac{1000 \mu\text{L}}{1 \mu\text{L}} = 10.42 \mu\text{L}$$

Start Time of Incubation: 11:55 am

End Time of Incubation: 12:55 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

Heat @ 65°C for 20 mins

Where did you store your finished product and what did you label it?

-20°C Freezer | 1 tag digest 4 R

NEXT STEP:

# Gel Electrophoresis

Page \_\_\_\_\_

Name: Josiah Riche / Ryan George

Date: 4/1/14

Time: 1:30 pm

% Agarose: 1

Lane Reagents

1

2

3

4

5

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8

|        |  |
|--------|--|
|        |  |
|        |  |
|        |  |
|        |  |
|        |  |
| tag    |  |
|        |  |
| ladder |  |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

- NEB Quick load 2 log ladder (6  $\mu$ L)
- tag - K215001 ~~m~~ digest product (5  $\mu$ L)
- 6x loading dye (1  $\mu$ L)

Start: 1:40pm @ 160V

End:



## Gel Electrophoresis

Name: JOSIAH RICKET + RYAN GEORGEDate: 7/1/21Time: ~~11:55 am~~ 1:30 pm

Picture

## Results

50 ~~um~~ L 1xTAC  
.5g agarose  
2.5 ul gel red

Digest failed. Got 1 band at 1300 bp.  
should have been 2 bands w/ 1st  
850 bp.

## NEXT STEP:

Liquid culture & troubleshoot

Name(s): Ryan GeorgeDate and Time: 4/11/14 9:10pmLiquid CultureAmount of LB used: 5 mLPart Used: K215001 (A2 resistant)Antibiotic Used: A2Amount of Antibiotic Used: 10  $\mu$ LConcentration of Antibiotic: 100  $\mu$ g/mLLabel on Product: K215001 Amp SRLocation of Product: 37°C shakerNext Step: M.M. prep

Name(s): Jacob Jodet  
Christa Winslow

Date and Time: 4/01/18 8:15pm

**Liquid Culture**

Amount of LB used: 5mL

Part Used: K314200 toxin gene

Antibiotic Used: chloramphenicol

Amount of Antibiotic Used: 2uL

Concentration of Antibiotic: 20uL/mL

Pat Liquid Culture

Label on Product: Igen part K314200

Location of Product: warm room at 8:30 pm

Next Step: miniprep

# Gel Electrophoresis

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Name: Jacob Soder, Christer Winslow

Date: 8:45 4/18/13

Time: 8:45 pm

% Agarose: 1.5% .75g agarose  
50 mL buffer 2.5 mL gel red  
Set gel out to cool at 8:15

Lane Reagents

1  
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4  
5  
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7  
8

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|  
|  
|  
|

5  $\mu$ L miniprep BBa\_K875001 + 1  $\mu$ L loading dye  
4  $\mu$ L 1kb DNA ladder + 1  $\mu$ L loading dye

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Next time use 1  $\mu$ L 1kb and .2-.5  $\mu$ L loading dye  
I can run the gel longer to get better separation of the bands  
ladder

Reagents used:

miniprep of BBa\_K875001

gel run started at 9:50pm  
finished at 10:35pm

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Picture

### Results

band at the 4<sup>th</sup> ladder band, so it is about 2000 bp  
~~band around 900 bp, but should have been at 2000 bp → 4<sup>th</sup>?~~

NEXT STEP:

Name(s): RYAN GEORGE  
JOSIAH RICEDate and Time: 4/2/14 2:00pmLiquid Culture

|   |                              |                               |
|---|------------------------------|-------------------------------|
| 1 | Amount of LB used:           | <u>5mL</u>                    |
| 2 | Part Used:                   | <u>001 <del>amp</del> tag</u> |
| 3 | Antibiotic Used:             | <u>chlcr</u>                  |
| 4 | Amount of Antibiotic Used:   | <u>1 uL</u>                   |
| 5 | Concentration of Antibiotic: | <u>10 ug/mL</u>               |

1. 5mL
2. 001 tag
3. amp
4. 10uL
5. 100ug/mL

|                      |                      |          |                    |
|----------------------|----------------------|----------|--------------------|
| Label on Product:    | <u>001 Tag chlcr</u> | <u>1</u> | <u>001 Tag amp</u> |
| Location of Product: | <u>37° Shaker</u>    |          |                    |
| Next Step:           | <u>Mini Prep</u>     |          |                    |

Date and Time: 4:55 PM 4/2/14 Performed by: Zach Biner**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB 2.1
- o DNA K215010, K215104
- o Enzyme SpeI, XbaI

| MATERIALS | TYPE    | AMOUNT USED |
|-----------|---------|-------------|
| Buffer    | NEB 2.1 | 2.5 ml      |
| DNA       | K215010 | 10 ml       |
| Enzyme(s) | SpeI    | 1.5 ml      |
|           | XbaI    | 1.5 ml      |
| Water     | DEPC    | 11.5 ml     |

Total Reaction Volume 25 ml

Construct a table with this format for each reaction that you do today.

|        |         |         |
|--------|---------|---------|
| Buffer | NEB 2.1 | 2.5 ml  |
| DNA    | K215104 | 10 ml   |
| Enzyme | SpeI    | 1.5 ml  |
|        | XbaI    | 1.5 ml  |
| Water  | DEPC    | 11.5 ml |

total: 25 ml

Start Time of Incubation: 5:48 pm

End Time of Incubation: 5:39 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes):

37° <sup>30 min</sup> → 80° <sup>20 min</sup>

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

iGEM plasmid box -20°C, initials, parts, date, resclis est

NEXT STEP:

run gel and if successful ligate



Mini Prep

Name(s): Darcy Todat  
Date and Time: 4/2/14 8:00pm

Check off as you complete the steps

Procedure:

Bacteria used: K314200

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1..... in 100 ☒  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: BBa\_K314200  
Location of product: Room Plasmid box

Deviations from Procedure and other Notes:

~~Light culture of BBa\_K314200 in  $-20^{\circ}\text{C}$~~

Successful?

NEXT STEP:

Gel electrophoresis

Continued on back? Yes ☐; No ☐

# Gel Electrophoresis

Page 91

Name: David Schradl

Date: 4/2/14

Time: 5:30/7:45

% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

|   |  |
|---|--|
|   |  |
| <del>K215000</del><br><del>Amplif</del> |  |
| <del>K215000</del><br><del>Amplif</del> |  |
| K2150100<br>R.O.                        |  |
| K215000 (a)<br>R.O.                     |  |
| K2151000<br>R.O.                        |  |
| K2151000<br>R.O.                        |  |
| K2151000<br>R.O.                        |  |
| Ladder                                  |  |

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

→ 2.5 ml Gel Red

50 ml 1xTAE

0.5 g Agarose

→ 5 ml 2x loading buffer (Koch stock)

→ 1 ml 6x loading dye to 5 ml samples

# Gel Electrophoresis

Name: Daniel Schaefer  
Date: 9/3/14  
Time: 4:40 PM

Picture

## Results

- Lanes 2 & 6 matched w/ lane 2 & also showed only one band (the bands total for each lane)
- Lanes 4 & 5 matched w/ lane 3 & also showed only one band (the bands total for each lane)

"we hypothesize"

we expect  
3 bands total  
for each lane  
which tells  
us one enzyme  
did not work  
for each  
remember we

NEXT STEP:

overexpressed  
the enzymes  
during the  
restriction  
digest

## Mini Prep

Name(s): Ryan George  
Date and Time: 4/14/14 2:50pm

Check off as you  
complete the steps

**Procedure:**

Bacteria used: K215001 C3

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant. ☒
2. Resuspend in 250 $\mu$ L of buffer P1. ☒  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2. ☒  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3. ☒  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm. ☒
6. Pipet supernatant into a QIAprep spin column. ☒  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column. ☒  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column. ☒  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube. ☒
10. Add 50 $\mu$ L Buffer EB to column. ☒  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through. ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use. ☒

Label on centrifuge tube: Tag mini prep RG  
Location of product:  $-20^{\circ}\text{C}$  freezer

Deviations from Procedure and other Notes:

Successful?

Yes (at least the Qubit was)

NEXT STEP:

Qubit & Digest

Continued on back? Yes ☐ No ☒

Date and Time: 4/4/14 3:35pm Performed by: Ryan George

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed 1
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 700 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 3.5
4. Subtract #3 from #2 696.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Name(s): Ryan GeorgeDate and Time: 4/6/14 5:30pmLiquid CultureAmount of LB used: 5mlPart Used: K215001 (A2 resistant)Antibiotic Used: AmpAmount of Antibiotic Used: 0ml, 2ul, 4, 6, 8Concentration of Antibiotic: 0mg/ml, 20mg/ml, 40, 60, 80

Done to see if this min. inhibitory concentration.

Label on Product: K215001 — mg/ml Amp RGLocation of Product: 37°C shaker (orange tape)Next Step: Observe results

Date and Time: 4/6/14 5:30pm Performed by: Ryan George**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer -20°C, BioBrich Assembly
- o DNA -20°C freezer, page 42
- o Enzyme Sf. Mol. bio lab freezer

| MATERIALS | TYPE       | AMOUNT USED          |
|-----------|------------|----------------------|
| Buffer    | NEB 2.1    | 5 $\mu$ L            |
| DNA       | K25001     | 9.5 $\mu$ L          |
| Enzyme(s) | SfiI, XbaI | 1 $\mu$ L, 1 $\mu$ L |
| Water     | DEPC       | 33.5 $\mu$ L         |

Total Reaction Volume 50  $\mu$ L

Construct a table with this format for each reaction that you do today.

$$1 \mu\text{g} \cdot \frac{1 \text{ mL}}{105 \mu\text{g}} \cdot \frac{1000 \mu\text{L}}{1 \text{ mL}} = 9.52 \mu\text{L}$$



Start Time of Incubation: 5:25 6:25 pm

End Time of Incubation: 7:25 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

Heat 20 mins at 65°C

Where did you store your finished product and what did you label it?

-20°C freezer, tag RG (blue)

NEXT STEP:

Gel electrophoresis & ligation (hopefully!)

# Gel Electrophoresis

Page \_\_\_\_\_

Name: Ryan George

Date: 4/6/14

Time: 7:30 pm

% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

digest

ladder

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

- 50 mL 1x TAE
- 0.5g agarose
- 2.5 mL gelred

Start: 7:56pm @ 100V  
end:

6µL Quickload ladder (2-log)

5µL digest product

1µL 6x loading dye

## Gel Electrophoresis

Name: Ryan George  
Date: 4/6/14  
Time: 7:30 pm

Picture

## Results

Failed for the 3rd time. Only 1 band @ 1300 bp.  
Should be two w/ 1 at 850 bp.

NEXT STEP:

Mini Prep  
 Name(s): Ryan George, Josiah Riche  
 Date and Time: 4/7/14 2:35pm

Check off as you  
complete the steps

**Procedure:**

Bacteria used: K215001 AmpR (80mg/mL broth)

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant. ☒
2. Resuspend in 250 $\mu$ L of buffer P1. ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2. ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3. ☒  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm. ☒
6. Pipet supernatant into a QIAprep spin column. ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column. ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column. ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube. ☒
10. Add 50 $\mu$ L Buffer EB to column. ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through. ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use. ☒

Label on centrifuge tube: 001 R 4/7/14 80mg/mL  
 Location of product:  $-20^{\circ}\text{C}$

## Deviations from Procedure and other Notes:

we used the 80mg/mL culture from page 94. 40mg/mL and 60mg/mL had growth. 0 and 20 mg/mL did not grow.

Successful?

NEXT STEP:

Qubit

Continued on back? Yes ☐; No ☐

Date and Time: 4/7/14 3:00pm Performed by: Ryan George, Josiah Riche

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 1
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 700 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 3.5
4. Subtract #3 from #2 696.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 8:20 pm 3/4/14 Performed by: JOSIAH RUCK, RYAN GEORGE  
JOSEPH LEVERENCE

### Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

### PROCEDURE

#### Material Location

- o Buffer biobrick assembly
- o DNA BBA-1C215001 Tag from Mini Prep (98)
- o Enzyme Xba I Spa I

| MATERIALS | TYPE                | AMOUNT USED                                    |
|-----------|---------------------|--|
| Buffer    | <u>2.1</u>          | <u>5 <math>\mu</math>L</u>                     |
| DNA       | <u>1C215001 TAG</u> | <u>43 <math>\mu</math>L</u>                    |
| Enzyme(s) | <u>Spa I Xba I</u>  | <u>1 <math>\mu</math>L 1 <math>\mu</math>L</u> |
| Water     | DEPC                | <u>0</u>                                       |

Total Reaction Volume 50  $\mu$ L  
~~50  $\mu$ L~~

Construct a table with this format for each reaction that you do today.

$$1 \mu\text{g DNA} \cdot \frac{1 \text{ mL}}{15.6 \mu\text{g}} \cdot \frac{1000 \mu\text{L}}{1 \text{ mL}} = 64.1$$

Start Time of Incubation: 8:50 pm

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°

Method used for Quenching the Reaction (*different for different enzymes*)

Heat 20 mins @ 65°C

Where did you store your finished product and what did you label it?

\_\_\_\_\_

NEXT STEP:

Gel electrophoresis ← *course that  
words spelling*



Date and Time: 4/9/14Performed by: Daniel Schmed**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB Buffer 2.1
- o DNA k215010, k215104
- o Enzyme Spe I, ~~Xba I~~ EcoR I

| MATERIALS | TYPE                             | AMOUNT USED          |
|-----------|----------------------------------|----------------------|
| Buffer    | NEB 2.1                          | 2.5 ml               |
| DNA       | k215010                          | 10 <del>0.5</del> ml |
| Enzyme(s) | Spe I<br><del>Xba I</del> EcoR I | 0.5 ml<br>0.5 ml     |
| Water     | DEPC                             | 11.5 <del>2</del> ml |

Total Reaction Volume 25 ml

Construct a table with this format for each reaction that you do today.

Trial 1: will use Spe I & ~~Xba I~~ EcoR I  
 Trial 2: will use Spe I (use 12 ml DEPC)  
 Trial 3: " " ~~Xba I~~ (use 12 ml DEPC) EcoR I

testing to see if there  
 is an issue with an enzyme

0.5

4.5

0.5

4.5

0.5

4.5

used 6 ml of DNA  
 because my really low  
 & 16 ml of DEPC

I realized this after  
 I got these ready

8.45 PM

\* Note: Realized we are changing  
 the aspect of this R.D.  
 from finding out why  
 one of the enzymes  
 was not working to  
 switching the ~~one~~  
 enzyme Xba I to  
 EcoR I

Note (9:10 PM) if one works and the other doesn't (for observing how many bands per lane) then we believe  $XbaI$  went wrong

Start Time of Incubation: 9:05 PM

End Time of Incubation: 9:35 PM

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (different for different enzymes)

20 min ; 80°C

Where did you store your finished product and what did you label it?

-20°C on green rack  
Freezer ; ~~16:00 PM~~ placed box labelled (14/11/12) 9/14/12

NEXT STEP:

run gel

# Gel Electrophoresis

Page \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: 1%

Lane Reagents

1

2

3

4

5

6

DNA

7

DNA

8

2log  
ladder

Reminders:

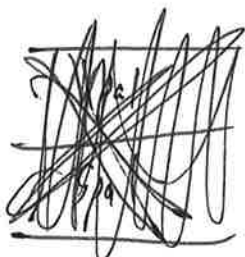
Add Gel Green

Add Loading Dye

Run Red

Reagents used:

K215001 post restriction  
digest



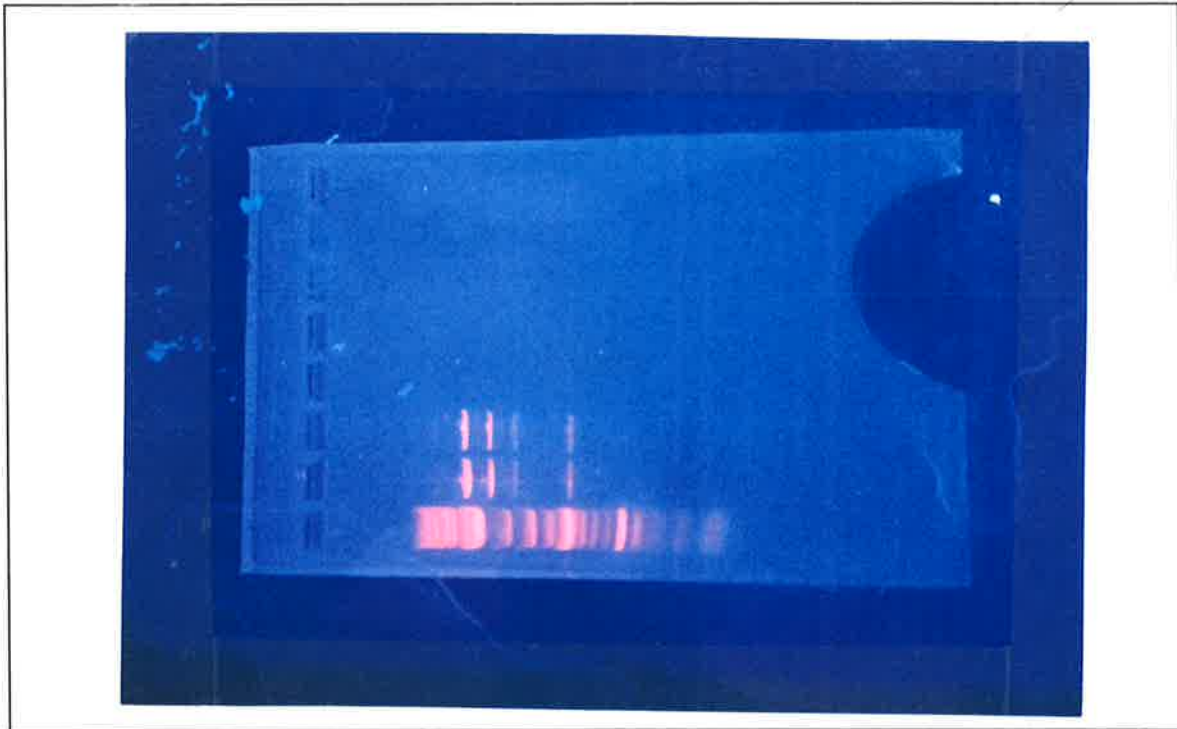
K215001 pre digest

NEB 2log quickload ladder

Restriction digest product from page 99

## Gel Electrophoresis

Name: Josh Leverence Ryan George  
Date: 4/9/14 Josiah Ricke  
Time: 8:55 pm



Results Digestion was SUCCESSFUL

NEXT STEP:

Ligation

# Gel Electrophoresis

Page 102

Name: Daniel G. [Signature]  
 Date: 5/10/14  
 Time: 5:15

% Agarose: 1

| Lane | Reagents                         | Results   |
|------|----------------------------------|---|
| 1    | K215004 (MMP-9)                  | →   |
| 2    | K215016 (I <sub>2</sub> ) - R.D. | →   |
| 3    | K215016 (I <sub>2</sub> ) - R.D. | → <del>2 bands</del> light 2 bands → 1st at farthest you can see lane 3 band and then the second band is a little further than that |
| 4    | K215016 (I <sub>2</sub> ) - R.D. | → 2 bands   |
| 5    | K215104 (I <sub>2</sub> ) - R.D. | → Same as 2   |
| 6    | K215000 (I <sub>2</sub> ) - R.D. | → 2 bands   |
| 7    | K215000 (I <sub>2</sub> ) - R.D. | → Same as 2   |
| 8    | K215000 (I <sub>2</sub> ) - R.D. | → 2 bands   |
| 9    | Ladder                           | → Ladder  |

Reminders:  
 Add Gel Green  
 Add Loading Dye  
 Run Red

## Reagents used:

- 2.5 ml Gel Red
- 50 ml 1X TAE buffer
- 0.5g Agarose
- 5 ml 2 log ladder (BioLabs stock)
- 1 ml 6x loading dye to 5 ml samples

(used 60000 spec)

## Gel Electrophoresis

Name: Daniel Schvach  
Date: 4/10/14  
Time: 4:15

Picture

Results

→ we believe ~~that~~ Spe I is having probs

NEXT STEP:

ligation

Name(s): JOSIAH RICKS  
RYAN GEORGEDate and Time: 4/14/14 8:45Liquid CultureAmount of LB used: 5mLPart Used: DHSα

Antibiotic Used: \_\_\_\_\_

Amount of Antibiotic Used: \_\_\_\_\_

Concentration of Antibiotic: \_\_\_\_\_

Label on Product: R DHSα ElectrocompetentLocation of Product: Warm RoomNext Step: Electroporation

# Gel Electrophoresis

Page 104

Name: Zach Binn

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

K215010

K215104

Ladder

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used: K215010, K215104, Gel red, loading dye, agarose, ladder (1kb)



# Gel Electrophoresis

Name: Zach Binner  
Date: 4/15/14  
Time: 4:50 PM

Picture

Results samples ran well, but I am not used to ~~gels~~ /adder that needs dye so I didn't add any so there is no ladder.

NEXT STEP:

Date and Time: 9/22/14 Performed by: Daniel Schroed**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- Buffer NEB Buffer 2.1
- DNA 304450, K1175001 → the plasmids we will ligate the secretion pump/tag into
- Enzyme Spe I, EcoRI

| MATERIALS | TYPE                        | AMOUNT USED      |
|-----------|-----------------------------|------------------|
| Buffer    | NEB 2.1                     | 2.5 ml           |
| DNA       | <del>304450</del><br>304450 | 10 ml            |
| Enzyme(s) | Spe I<br>EcoRI              | 0.5 ml<br>0.5 ml |
| Water     | DEPC                        | 11.5 ml          |

Total Reaction Volume 25 ml

also another  
restriction  
enzyme digest  
w/ K1175001

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 4:20 PM

End Time of Incubation: 4:50 PM

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

20 min; 80°C

Where did you store your finished product and what did you label it?

Freezer, smell green, frag methyl R.D. 4/8/14 4/22/14

NEXT STEP:

Run gel & if we got  
good results ligate next

Date and Time: 4-23-14

Performed by: Matt Mortenson Anna Gorney

**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer Neb 2.1
- o DNA Yes Z, XynA, BglI purified PCR product (p60, p53, p<sup>60</sup>)
- o Enzyme XbaI, ~~BglI~~ SpeI

| MATERIALS | TYPE                 | AMOUNT USED                                |
|-----------|----------------------|--|
| Buffer    | NE Buffer            | <del>0</del> <sup>5</sup> <del>20</del> uL |
| DNA       | purified PCR product | 40 uL                                      |
| Enzyme(s) | XbaI<br>SpeI         | 2.5 <del>10</del> x2                       |
| Water     | DEPC                 | 0  |

Total Reaction Volume <sup>50</sup> ~~100~~ uL

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

\_\_\_\_\_

Where did you store your finished product and what did you label it?

PCR Box

NEXT STEP:

Name(s):

Anna

Date and Time:

Liquid Culture

Amount of LB used: 5mL / 5mL

Part Used: mCherry / gmpF E.coli

Antibiotic Used: Kanamycin

Amount of Antibiotic Used: 3.5uL

Concentration of Antibiotic: 35ug/mL

Label on Product: mCherry / E.coli gmpF

Location of Product: warm room

Next Step: plate

Name(s): RYAN GEORGE  
JOSIAH RICKSDate and Time: # 4/23/14 11:15pmLiquid CultureAmount of LB used: 5mLPart Used: DH5 $\alpha$ Antibiotic Used: ✓Amount of Antibiotic Used: ✓Concentration of Antibiotic: ✓Label on Product: JR DH5 $\alpha$  ElectrocompetentLocation of Product: Warm RoomNext Step: Electroporation

# Gel Electrophoresis

Name: Daniel Schoedt  
 Date: 4/24/19  
 Time: 3:58 PM

% Agarose: 1

Lane Reagents

|   |                 |  |
|---|-----------------|--|
| 1 | —               |  |
| 2 | —               |  |
| 3 | —               |  |
| 4 | K117501 Plasmid |  |
| 5 | Tomato Plasmid  |  |
| 6 | K117501 Rb      |  |
| 7 | Tomato Rb       |  |
| 8 | Ladder          |  |

Reminders:  
 Add Gel Green  
 Add Loading Dye  
 Run Red

Left of bands they appear to be in different places from lane 7 to lane 6

show strong band but these smaller bands (band corresponds from lane 7 to 5 & lane 6 to 4)  
 plasmids went in gel well

Reagents used:

gel { 0.5g agarose  
 50ml TAE buffer  
 2.5ml Gel Red

samples { 5ml DNA  
 1ml loading dye

ladder { 6ml ladder



## Gel Electrophoresis

Name: Daniel Schach  
Date: 4/24/19  
Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

Ligation

# Gel Electrophoresis

Page 110

Name: Zach Biner

Date: 4/24/14

Time: 8:00 PM

% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

215010

215104

ladder 1kb

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

Parts above + gel red + loading dye.

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

found out the ones I ran were cut wrong

## Gel Electrophoresis

Name: Zach BirnerDate: 4/25/14Time: 2:45% Agarose: 1

Lane Reagents

|   |         |
|---|---------|
| 1 |         |
| 2 |         |
| 3 |         |
| 4 | K117504 |
| 5 | J04450  |
| 6 | 215010  |
| 7 | 215104  |
| 8 | Ladder  |

Reminders:  
 Add Gel Green  
 Add Loading Dye  
 Run Red

## Reagents used:

K215104 (spcl, EcoRI T<sub>1</sub>)K215010 ( " " T<sub>1</sub>)

J04450 ( " " )

K117500 ( " " )

Ladder (Kb)

Loading dye

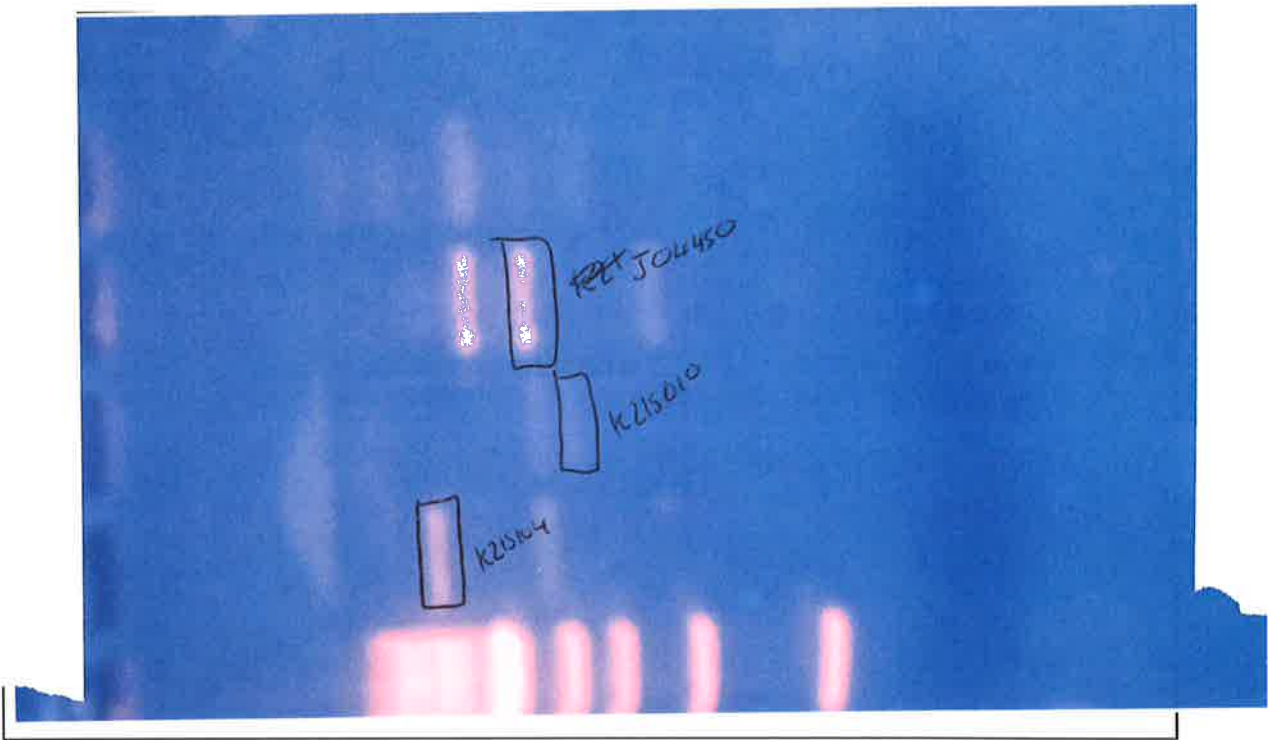
Gel reel

## Gel Electrophoresis


Name: Zach Binner

Date: \_\_\_\_\_

Time: \_\_\_\_\_



Results

 = to be extracted

NEXT STEP:

Extraction

Name(s): Mathias MortensenDate and Time: 4-25-14 5pmLiquid CultureAmount of LB used: 5 mLPart Used: BBA- K215002Antibiotic Used: AmpAmount of Antibiotic Used: 10  $\mu$ LConcentration of Antibiotic: 100  $\frac{\mu\text{g}}{\text{mL}}$ 

2 LCS - "Pump" part

Label on Product: K215002 Amp MWM 2-25-14Location of Product: Shaker PlateNext Step: Miniprep

Date and Time: 4/25/14 / 4:30 PM Performed by: Zach Birner

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |              |              |              |  |  |  |  |  |  |
|-------------------|--------------|--------------|--------------|--|--|--|--|--|--|
| Sample            | K215104      | K215010      | J04450       |  |  |  |  |  |  |
| Tube + Gel weight | 1.19g        | 1.15g        | 1.18g        |  |  |  |  |  |  |
| Empty tube weight | 1.06g<br>77g | 1.06g<br>77g | 1.06g<br>77g |  |  |  |  |  |  |
| Gel Weight        | .13g         | .09g         | .12g         |  |  |  |  |  |  |
| Buffer QG         | 340ul        | 270ul        | 360ul        |  |  |  |  |  |  |
| Isopropanol       | 130ul        | 90ul         | 120ul        |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

such as  
K215010

Label on Product: Extraction, "Part", ZB, 4/25/14  
 Location of Product: -20°C freezer in the Plasmid box  
 Next Step: ligation

Date and Time: 4/14/14 8:45pm  
 Protocol: Ligation  
 Reagents: see below

Lab Technicians(s) involved: ~~RYAN GEORGE~~  
JOSEPH RICE

Procedure (with applicable notes):

7  $\mu$ L ~~DTF~~ 001 From page 99  
 1  $\mu$ L T4 DNA Ligase  
 1  $\mu$ L Ligase Buffer  
 1  $\mu$ L ~~DTF~~ cells  $\leftarrow$  Pump cells  
 1  $\mu$ L xynA - unnecessary

Ideally do a  
3:1 ratio  
for next time

Results:

need to electroporate

Location of product: \_\_\_\_\_  
 Label on product: ~~DTF~~ Lig JR p103  $\rightarrow$  ~~DTF~~  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Electroporation

Continued on back? Yes ☐; No ☐



Name(s): Joiah Ricket  
Sierra Tackett

## Electroporation

Page: 115  
104

Reagents: \_\_\_\_\_

### Cell Preparation

Competent cells thawed? Yes ☒ No \_\_\_\_\_ Amount? \_\_\_\_\_

### Electroporation

Electroporation cuvette chilled? Yes ☒ No \_\_\_\_\_

Amount of SOC added to culture tubes: .1 mL  
→ use LB instead

DNA placed in m/f tubes? Yes ☒ No \_\_\_\_\_

Placed on ice? Yes ☒ No \_\_\_\_\_

Amount of DNA mixed with competent cells: \_\_\_\_\_

Voltage used for electroporation: Preset

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No \_\_\_\_\_

Amount added: .1 mL

Cells shaken at 37°C? Yes ☒ No \_\_\_\_\_

Start time: \_\_\_\_\_ End time: \_\_\_\_\_

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells spun in centrifuge (10-15s)? Yes \_\_\_\_\_ No \_\_\_\_\_

s/n decanted off? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells resuspended? Yes \_\_\_\_\_ No \_\_\_\_\_

Amount of solution: \_\_\_\_\_

Dilutions performed:

10<sup>-1</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-2</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-3</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-4</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-5</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-6</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-7</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-8</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

10<sup>-9</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: R Pump + Tag Post electroporation pg 104

Location of products: Warm Room

Next steps: Get ~~electroporation~~ / Liquid Culture

Signature: [Signature]

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

## Mini Prep

Name(s): Matthew Mortensen

Date and Time: 4-26-14 10:30am

Check off as you  
complete the steps

### Procedure:

Note: Zymo kit used

Bacteria used: BBa-K215002 (p112) (2 5mL cultures)

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant.
2. Resuspend in  $250\mu\text{L}$  of buffer P1.  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu\text{L}$
3. Add  $250\mu\text{L}$  Buffer P2.  
Invert 4-6 times (less than 5 minutes until next step)
4. Add  $350\mu\text{L}$  Buffer N3.  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm.
6. Pipet supernatant into a QIAprep spin column.  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add  $500\mu\text{L}$  Buffer PB to column.  
Centrifuge 30-60sec and then discard flow through
8. Add  $750\mu\text{L}$  of Buffer PE to column.  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube.
10. Add  $50\mu\text{L}$  Buffer EB to column.  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through.
12. Label and store in  $-20^{\circ}\text{C}$  for later use.

Used 600ul  
sterile H<sub>2</sub>O

100 ul lysis  
Buffer

Neutralization Buffer

2004L  
Endoverh

400 uL 2:92V  
Wash Buffer

30uL Elution Buffer

Label on centrifuge tube: BBa K215002 Plasmid MW/m 4-25 p116

Location of product: Plasmid Box

Deviations from Procedure and other Notes: Zymo "Zippy" Miniprep kit used. Changes to the protocol are noted on the left.

30  $\mu$ L Elution

### Successful?

NEXT STEP: Restriction Enzyme Digest.

Continued on back? Yes

☐; No ☒

Date and Time: 4-26-14 1:30 pm Performed by: Matt Mortensen Anna Garvey

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 4
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1300 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 6.5
- Subtract #3 from #2 1293.5
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Name(s): JOSIAH RICEDate and Time: 4/26/14Liquid CultureAmount of LB used: 5 mLPart Used: K215001Antibiotic Used: AmpAmount of Antibiotic Used: 10 mLConcentration of Antibiotic: 100 µg/mLx 2Amount of LB used: 5 mLPart Used: K215002Antibiotic Used: AmpAmount of Antibiotic Used: 10 mLConcentration of Antibiotic: 100 µg/mLx 2Label on Product: K215001 (1)(2) K215002 (1)(2)Location of Product: Warm Room

Next Step: \_\_\_\_\_

## PCR

Date and Time: <sup>4</sup> 26-14 3pm Performed by: Matthew Mortensen

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer 2x Bullseye Taq 2x Master Mix 12.5 uL  
 dNTPs MM  
 Forward Primer 1. Fwd Yes 2. Fwd XynA 3. BglI Fwd (PCR Box) 1.25 uL  
 Reverse Primer 1. Rev Yes 2. Rev XynA 3. BglI Rev (PCR Box) 1.25 uL  
 Template DNA 1. Yes 2. PCR Prod (p68) 3. XynA PCR Prod (p52) 4. BglI PCR Prod (p60) 10 uL  
 Taq DNA Polymerase MM  
 Nuclease-free water Dep C

## Procedure:

| Symbol on lid | Content in PCR tube   |
|---------------|---|
| V!            | 12.5 uL Master Mix 1.25 Fwd Yes 2 1.25 Rev Yes 2 10 uL Yes 2 PCR Prod |
| X!            | " " 1.25 Fwd XynA 1.25 Rev XynA 10 uL XynA PCR Prod                   |
| B!            | " " 1.25 Fwd BglI 1.25 Rev BglI 10 uL BglI PCR Prod                   |
|               |   |
|               |   |
|               |   |
|               |   |

## Temperature Settings

|              |       |
|--------------|-------|
| Denaturation | 95 °C |
| Annealing    | 63 °C |
| Extension    | 72 °C |
| Final        | 72 °C |

Number of cycles: 34

Time of completion: 5:35

Label on product(s): (Gene) PCR Amplification num p 117

Location of product(s) PCR Box

End notes/comments:

## NEXT STEP:

Restriction Digest

Continued on back? Yes ☐; No ☐

Date and Time: 4-26-14 Performed by: Anna Garvey**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB 2.1
- o DNA K215002
- o Enzyme NheI

| MATERIALS | TYPE    | AMOUNT USED           |
|-----------|---------|-----------------------|
| Buffer    | NEB2.1  | 5ul                   |
| DNA       | K215002 | 1.5ul                 |
| Enzyme(s) | NheI    | 1.5ul                 |
| Water     | DEPC    | <del>47</del> at 42ul |

Total Reaction Volume 50ul

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 2:50

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 65°C

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

NEXT STEP:



Date and Time: 4-28-14Performed by: Matt Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 3
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1100
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 5.5
4. Subtract #3 from #2 1094.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 4-28-14 Performed by: MAT ANNA GARVEY**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB 2.1
- o DNA X40A
- o Enzyme XbaI, SpeI

| MATERIALS | TYPE       | AMOUNT USED                                 |
|-----------|------------|---|
| Buffer    | NEB 2.1    | 2.5ul                                       |
| DNA       | X40A       | <del>12.5ul</del> 16.1                      |
| Enzyme(s) | XbaI, SpeI | 1.5ul of each                               |
| Water     | DEPC       | 9.4 <del>12.5</del> (low DNA concentration) |

Total Reaction Volume 25ul

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 8:00pm

End Time of Incubation: 9:00pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 65°

Method used for Quenching the Reaction (*different for different enzymes*)

0

Where did you store your finished product and what did you label it?

1.01

2.4

NEXT STEP:

Date and Time: 4-28-14 Performed by: ANNA GARUCY**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- Buffer NEB 2.1
- DNA YesZ
- Enzyme Xba I, Spe I

| MATERIALS | TYPE         | AMOUNT USED  |
|-----------|--------------|--------------|
| Buffer    | NEB 2.1      | 2.5 $\mu$ L  |
| DNA       | YesZ         | 5.32 $\mu$ L |
| Enzyme(s) | Xba I, Spe I | 1.5 of each  |
| Water     | DEPC         | 14.18        |

Total Reaction Volume 25  $\mu$ L

Construct a table with this format for each reaction that you do today.

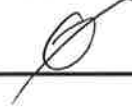
Start Time of Incubation: 8:00pm

End Time of Incubation: 9:00pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 65°

Method used for Quenching the Reaction (*different for different enzymes*)



Where did you store your finished product and what did you label it?

---

NEXT STEP:

Date and Time: 4-28-14 Performed by: MATT MORTENSEN + ANNA GARVEY**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB.2.1
- o DNA ~~XXXX~~ bglI
- o Enzyme XbaI, SpeI

| MATERIALS | TYPE                 | AMOUNT USED   |
|-----------|----------------------|---------------|
| Buffer    | NEB2.1               | 2.5uL         |
| DNA       | <del>XXXX</del> bglI | 3.85uL        |
| Enzyme(s) | XbaI, SpeI           | 1.5uL of each |
| Water     | DEPC                 | 15.5uL        |

Total Reaction Volume 25uL

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 8:00pm

End Time of Incubation: 9:00pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 65°

Method used for Quenching the Reaction (*different for different enzymes*)



Where did you store your finished product and what did you label it?

NEXT STEP:



Date and Time: 7/29/14Performed by: Daniel Schaefer

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 3
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L  $5 \times 200 = 1000 \rightarrow 1000 + 100 = 1100 \mu\text{L}$ 
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200  $550 \mu\text{L} \div 200 = 2.75$   $1100 \mu\text{L} \div 200 = 5.5 \mu\text{L}$
- Subtract #3 from #2  $550 - 2.75 = 547.25$   $1100 - 5.5 = 1094.5 \mu\text{L}$
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA ~~BR~~ Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA ~~BR~~ Reagent <sup>H<sub>2</sub>O</sup>
- Vortex lightly to mix. <sup>H<sub>2</sub>O</sup>

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

|     |                       |                                  |
|-----|-----------------------|----------------------------------|
| K4: | <u>1.25 ng/mL</u>     | <u>0.25 ng/mL</u>                |
|     | <del>0.52 ng/mL</del> | in sample <del>0.10 ng/mL</del>  |
| K0: | <u>0.79 ng/mL</u>     | <u>0.16 ng/mL</u>                |
| J:  | <u>1.22 ng/mL</u>     | <u>0.243 ng/mL</u>               |
|     | <del>2.07 ng/mL</del> | in sample <del>0.414 ng/mL</del> |

## Results

[illegible]

Date and Time: 4/12/14  
4/15/14 4:15pm  
 Protocol: Ligation  
 Reagents: 10x T4 DNA Ligation buffer,  
Nuclease-free water (Qiagen),  
T4 DNA Ligase

Lab Technicians(s) involved: Amel El Guedd  
Zach Birner

### Procedure (with applicable notes):

|  |  |
|--|--|
| <p>Vector DNA: <u>2000</u><br/> <del>1000</del></p> <p>Insert DNA: <u>k21010</u><br/> <u>k215104</u></p> | <p>10ml<br/>         10ml</p> <p>* we are not adding Nuclease Free water due to our low yield during the Orbit step → instead we are adding up to 20ml <sup>wt</sup>our sample DNA</p> <p>* if it does not work, we can do PCR next to try to get more</p> |
|--|--|

### Results:

Location of product: Freezer ; plasmid box (labeled w/ date & ligation k215104 & k21010)  
 Label on product: green tube (only tube) 20 & 15  
 End Notes/Comments: if it works, transform 1-5ml of reaction into 50ml competent cells

### NEXT STEP:

run gel to observe if ligation works; if not, run PCR on stuff we have to get more of it; if it does work then transform into cells

Continued on back? Yes ☐; No ☐

# Gel Electrophoresis

Page 125

Name: Zach Bimer

Date: 4/30/14

Time: 07:50 PM

% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

| K215010

| K215104

| Ladder

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

failed

NEXT STEP:

go back <sup>to plasma</sup> and check concentration

## PCR

Date and Time: 4/30/14 8:00pm Performed by: Jacob Jolat, Matt Mortensen

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer In master mixdNTPs In master mixForward Primer A and C primersReverse Primer B and D primersTemplate DNA K875001, K314200Taq DNA Polymerase In master mixNuclease-free water DepC water

## Procedure:

Followed the NEB procedure  
and used bullseye master mix instead of NEB

| Symbol on lid | Content in PCR tube   |
|---------------|---|
| A             | <sup>(K875001)</sup><br>0.5uL A primer, 0.5uL B primer, 2uL template, 12.5uL master mix, 9.5uL nuclease free H <sub>2</sub> O |
| B             | 0.5uL A <sup>(K875001)</sup> , 0.5uL B, 12.5uL master, 11.5uL H <sub>2</sub> O  |
| C             | 2uL template, 12.5uL master, 10.5uL H <sub>2</sub> O  |
| D             | 0.5uL C primer, 0.5uL D primer, 2uL <sup>(K314200)</sup> template, 9.5uL nuclease free H <sub>2</sub> O, 12.5uL master        |
| E             | 0.5uL C primer, 0.5uL D primer, 12.5uL <sup>(K314200)</sup> template master, 11.5uL H <sub>2</sub> O                          |
| F             | 2uL <sup>(K314200)</sup> template, 12.5uL master, 10.5uL H <sub>2</sub> O   |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 60°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34Label on product(s): A, B, C, D, E, F

End notes/comments:

Time of completion: 9:08 pm  
11:15 pm

Location of product(s)

store in -20°C once cycle is done

In PCR box 126 on top of microcentrifuge tubes  
↑  
page #

NEXT STEP:

Continued on back? Yes ☐; No ☐







# Gel Electrophoresis

Page 128

Name: Sacob Sobot, Christa Winslow

Date: 5/1/14

Time: 8:30 pm

% Agarose: 1.5%

.75g agarose ~~128~~  
~~50ml Buffer~~ ~~loading dye~~  
2.5 ml gel red

Lane Reagents

1

2

3

4

5

6

7

8

2.5 mL F + .5 mL loading dye

2.5 mL E + .5 mL dye

2.5 mL D + .5 mL dye

2.5 mL C + .5 mL dye

2.5 mL B + .5 mL dye

2.5 mL A + .5 mL dye

Ladder + .5 mL dye  
1 mL

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Gel started at 8:44 pm → 10:15

Reagents used:

See page 126

A =

1875001 2 mL, 12.5 mL mastermix, .5 mL A primer, .5 mL B primer, 9.5 mL <sup>Dep C</sup> ~~mastermix~~ H<sub>2</sub>O

(no template) B =

.5 mL primer A, .5 mL primer B, 12.5 mL mastermix, 10.5 mL H<sub>2</sub>O Dep C

(no primer) C =

~~1875001~~ 2 mL 1875001, 12.5 mL master, 10.5 mL H<sub>2</sub>O

D =

1875001 2 mL, .5 mL C primer, .5 mL D primer, 12.5 mL mastermix, 9.5 mL DepC water

(no template) E =

.5 mL C primer, .5 mL D primer, 12.5 mL mastermix, 9.5 mL DepC H<sub>2</sub>O

(no primers) F =

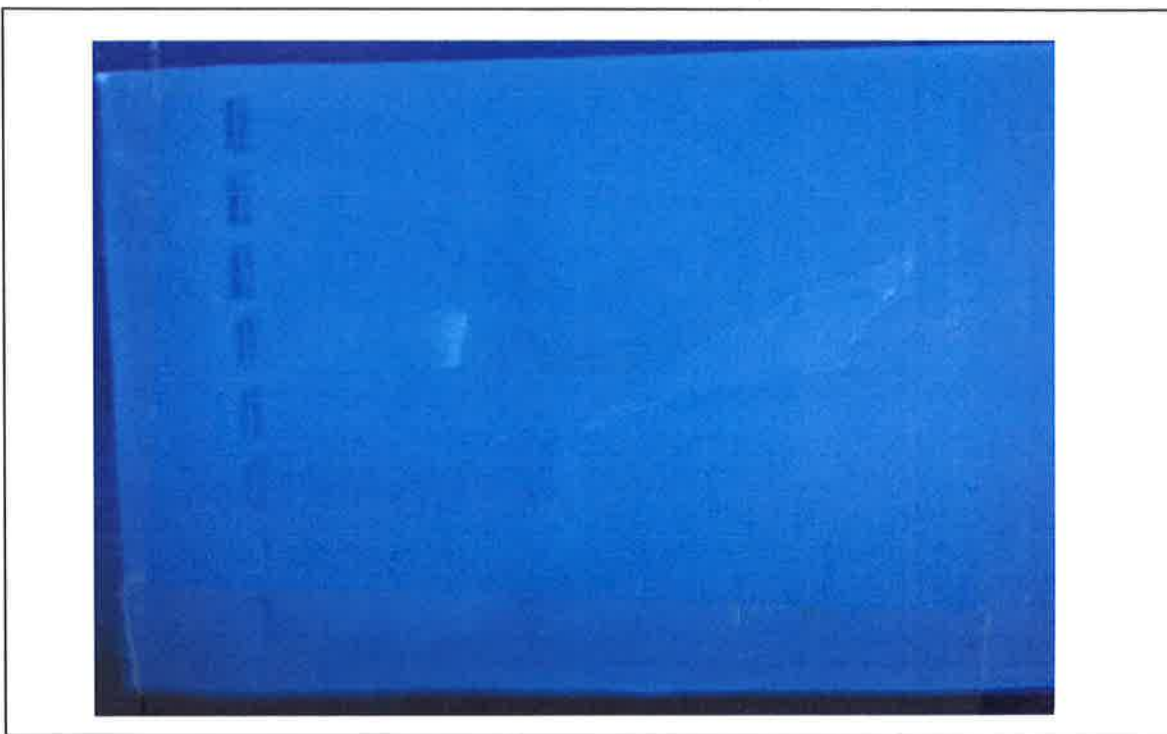
2 mL 1875001, 12.5 mL mastermix, 10.5 mL DepC H<sub>2</sub>O

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_



Results There are bands but they are very faint  
ladder was very faint  
The band at D → more distinct  
Band at 4 → beyond loading dye  
Band at B →

NEXT STEP:

Qubit

Date and Time: 5-2-14 5:30 Performed by: Matthew Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 6
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1700 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 7.5
4. Subtract #3 from #2 1692.5 $\mu$ L
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

| Sample Name                    | Page | Concentration      |
|--------------------------------|------|--------------------|
| PCR Prod A                     | p126 | 8.65 ug/mL         |
| B Negative for A (No Template) | p126 | 3.20 ug/mL         |
| C Negative for B (No Primers)  | p126 | 2.61 ug/mL         |
| D                              | p126 | 58.1 ug/mL         |
| E Negative for D (No Template) | p126 | 2.37 ug/mL         |
| F Negative for D (No Primers)  | p126 | Too low 2.02 ug/mL |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |
|                                |      |                    |

PCR using sewing primers A and B (Product A) had a lower concentration of DNA than expected, although not low enough to conclusively call it a failed PCR (Note that products B and C have a lower concentration when summed than PCR A alone; ~~therefore~~ PCR A and PCR B+C should have the same amount of genetic material. The status of reaction A is inconclusive.

Reaction D was successful as also evidenced by the agarose gel (p128).

## PCR

Date and Time: 5-2-14 5:45

Performed by: Matthew Morison

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer Mid Sci. Bulky 2x PCR Master Mix 12.5  $\mu$ L  
 dNTPs MM  
 Forward Primer Safety Team Sewing PCR Primer A 1.25  $\mu$ L  
 Reverse Primer Safety Team Sewing PCR Primer B 1.25  $\mu$ L  
 Template DNA BBa\_K875001 Plasmid Isolation (p30) or PCR Product A (p126) 5  $\mu$ L  
 Taq DNA Polymerase MM  
 Nuclease-free water Dep C 5  $\mu$ L

Procedure:

| Symbol on lid  | Content in PCR tube  |
|----------------|--|
| AB             | "12.5 MM" "1.25 $\mu$ L Primer A 1.25 $\mu$ L Primer B" 5 $\mu$ L 875001 Plasmid 5 $\mu$ L Dep C |
| P              | " " " " " 5 $\mu$ L PCR Product A 5 $\mu$ L Dep C  |
| N <sub>1</sub> | " " " " " — 7.5 $\mu$ L Dep C  |
| N <sub>2</sub> | " " " " " 5 $\mu$ L 876001 Plasmid 7.5 Dep C   |
|                |  |
|                |  |
|                |  |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 63°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: 4:45

Label on product(s): Part 875001 PCR <sup>AB</sup> <sub>N<sub>1</sub></sub> MM, 130

Location of product(s) PCR Box

End notes/comments:

NEXT STEP:

Continued on back? Yes ☐; No ☐



Date and Time: 7:20PM 8/2/14 Performed by: Zach Biner

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed 4
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1300
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 6.5
4. Subtract #3 from #2 1293.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]



Date and Time: 8:05 PM 5/2/14 Performed by: Zach Biner

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 5 + 4
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1100, 900
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 5.5 + 4.5
- Subtract #3 from #2 1094.5 + 895.5
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 5-2-14 8:50

Performed by: Matthew Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 4

- Note: You will need enough master mix for two more (The standards)

~~2.~~ Take #1 and add two samples, then multiply this by 200  $\mu$ L and add an extra 100  $\mu$ L ~~+300  $\mu$ L~~

- Note: the extra 100  $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.

~~3.~~ Take #2 and divide it by 200 ~~6.5  $\mu$ L~~

895.6 Quant-iT + 4.5  $\mu$ L Quant-iT  
Buffer Reagent

~~4.~~ Subtract #3 from #2 ~~+293.5  $\mu$ L~~

5. Find a tube capable of holding #2 worth of liquid

- Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.

6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.

7. Add #3 of 200X Quant-iT dsDNA BR Reagent

8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190  $\mu$ L Master Mix with 10  $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.

2. Repeat Step #1 for Quant-iT BR Standard #2

- Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
- Note: The standards should be in the 4°C
- Note: Only write on the caps of Qubit tubes when labeling them

3. For each of your samples in individual Qubit tubes, mix 199  $\mu$ L master mix with 1  $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"

2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.

3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1  $\mu$ L; record the number you get.

4. Repeat step #3 for each sample

5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Name(s): Zach BirnerDate and Time: 9:00pm 5/2/14Liquid Culture

Inoculum: A1

A3

C4

Amount of LB used: 5ml eachPart Used: K215104, K215010, K215304405Antibiotic Used: AmpAmount of Antibiotic Used: 10ml eachConcentration of Antibiotic: 100mg/ml

\$ Plt in AT 9:25 PM

Label on Product: Part, ZB, Date, AMPLocation of Product: warm roomNext Step: mini prep

## Gel Electrophoresis

Name: Matthew MortensenDate: 5-2-14Time: 8:50 - 9:55% Agarose: 1%

Lane Reagents

|   |                |  |   |
|---|----------------|--|---|
| 1 | <del>W</del>   |  | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | L              |  |   |
| 3 | AB             |  |   |
| 4 | P              |  |   |
| 5 | N <sub>1</sub> |  |   |
| 6 | N <sub>2</sub> |  |   |
| 7 |                |  |   |
| 8 |                |  |   |

Reagents used:

L = 1kb ladder 1uL + 1uL Loading Dye

AB = 2.5uL PCR Product AB + .5uL Loading Dye

P = 2.5uL PCR Product P + .5uL Loading Dye

N<sub>1</sub> = 2.5uL PCR Product N<sub>1</sub> + .5uL Loading DyeN<sub>2</sub> = 2.5uL PCR Product N<sub>2</sub> + .5uL Loading Dye

## Gel Electrophoresis

Name: Matthew Mortensen

Date: 5-2-14

Time: \_\_\_\_\_



Results Only ~~Primers~~ <sup>Primer</sup> and Template DNA visualized indicating PCR failed.

The template DNA appeared smeared in both instances of its use ( $N_2$  and AB)  
Plasmid

NEXT STEP: Liquid Culture BBa-K876001

Name(s): Matthew MortensenDate and Time: 5/2/14Liquid CultureAmount of LB used: 5mLPart Used: BB-1875001Antibiotic Used: ChloroAmount of Antibiotic Used: 10uLConcentration of Antibiotic: 10ug/mL

x2

Label on Product: Chloro BB-1875001 MWM 5/2/14Location of Product: Warm Room ShakerNext Step: Miniprep



## Zymo Mini Prep

Name(s): ANNA GARVEY  
Date and Time: 5-3-14

Check off as you  
complete the steps

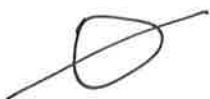
**Procedure:**

Bacteria used: V3875001

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒
- Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☒
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☒
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: V3875001  
Location of product: -20 PCR Box

Deviations from Procedure and other Notes:



NEXT STEP:

Qubit

Continued on back? Yes ☐; No ☒

Date and Time: 5-3-14 Performed by: ANNA GARVEY

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Name(s): Matthew MortensonDate and Time: 5/3/14 12:20 pmLiquid CultureAmount of LB used: 6 mLPart Used: K215010, K215104, J04450Antibiotic Used: Amp, Amp, KanAmount of Antibiotic Used: 10  $\mu$ L, 10  $\mu$ L, 3.5  $\mu$ LConcentration of Antibiotic: 100  $\mu$ g/mL, 100  $\mu$ g/mL, 35  $\mu$ g/mL

x2

Put in at 1 pm

Label on Product: <Part> <Antibiotic> mwm 5-3-14Location of Product: Shaker PlateNext Step: Miniprep

## PCR

Date and Time: 5-3-14 12:33pmPerformed by: ANNA GARVEY

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer MidSci Bullseye 2x PCR Master MixdNTPs in MMForward Primer Safety Team Sewing PCR Primer AReverse Primer Safety Team Sewing PCR Primer BTemplate DNA BBo-KB75001 pp.137Taq DNA Polymerase in MMNuclease-free water Dep C

12.5uL

1.25uL

1.25uL

5uL

5uL

Procedure:

| Symbol on lid  | Content in PCR tube  |
|----------------|--|
| AB             | 12.5uL + 1.25uL Primer A + 1.25uL Primer B + 5uL B75001 plasmid + 5uL DepC |
| N <sub>1</sub> | No Plasmid → changes 10uL DepC   |
| N <sub>2</sub> | No Primers → changes 7.5 DepC  |
|                |  |
|                |  |
|                |  |
|                |  |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 63°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) PCR Box

End notes/comments:

NEXT STEP:

Run Gel

Continued on back? Yes ☐; No ☐

# Gel Electrophoresis

Page 141

Name: Matthew Mortenson

Date: 5/13/14

Time: 3:15

% Agarose: 1%

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

L

AB

N<sub>1</sub>

N<sub>2</sub>

Reminders:

Add Gel Green

Add Loading Dye

Run Red

started at 3:20 @ 100V

Reagents used:

L = 1kb ladder (NEB) + 0.5 Loading dye

AB = PCR Product AB 2.5uL + 0.5LD (p140)

N<sub>1</sub> = PCR Product N<sub>1</sub> 2.5uL + 0.5LD (p140)

N<sub>2</sub> = PCR Product N<sub>2</sub> 2.5uL + 0.5LD (p140)

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: 5-3-14

Time: \_\_\_\_\_

Picture

Results PCR looks to have failed, although due to the very close sizes of the Primers and the PCR Product (potential PCR Product) a Qubit should be done to compare the amounts of DNA in the reaction and the negatives.

NEXT STEP:

Qubit

Date and Time: 5-3-14 4:02pm Performed by: Matthew Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

Name(s): ANNA GARVEY  
Matthew MortensenDate and Time: 5-3-14 4:45pmLiquid CultureAmount of LB used: 5mLPart Used: K215010, K215104, J04450Antibiotic Used: Amp, Amp, KanAmount of Antibiotic Used: 10 $\mu$ L, 10 $\mu$ L, 3.5 $\mu$ LConcentration of Antibiotic: 100 $\mu$ g/mL, 100 $\mu$ g/mL, 35 $\mu$ g/mL

Put in @ 4:30pm

Label on Product: <Part> <Antibiotic> AG 5-3-14Location of Product: Shallor PlateNext Step: Miniprep

## PCR

Date and Time: 5-3-14 5:10Performed by: ANNA GARVEY

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer MidSci Bullseye 2x PCR Master MixdNTPs MMForward Primer Safety Team PCR Primer AReverse Primer Safety Team PCR Primer BTemplate DNA Bba-K 875001 pp.137Taq DNA Polymerase MMNuclease-free water DepC

12.5ul

1.25ul

1.25ul

5ul

5ul

Procedure:

| Symbol on lid  | Content in PCR tube  |
|----------------|--|
| AB             | <sup>MM</sup> 12.5ul + 1.25ul Primer A + 1.25ul Primer B + 5ul B75001 + 5ul DepC |
| N <sub>i</sub> | No plasmid → 10 ul DepC  |
| N <sub>a</sub> | No primers → 7.5ul DepC  |
|                |  |
|                |  |
|                |  |
|                |  |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 55°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) \_\_\_\_\_

End notes/comments:

NEXT STEP:

Continued on back? Yes ☐ ; No ☐

Name(s): Anna Garvey  
Matt MortensenDate and Time: 5-3-14 7:15Liquid CultureAmount of LB used: 5mLPart Used: K875001, K215004, K215001Antibiotic Used: Chloro, Amp, AmpAmount of Antibiotic Used: 1.0ul, 10.0ul, 10.0ulConcentration of Antibiotic: Chloro:  $10^4$ g/mL  
Amp:  $100^4$ g/mL

Put in @ 7:30pm

Label on Product: <Part> <Antibiotic> MWM 5-3-14Location of Product: Warm Room - Shaker PlateNext Step: Mini Prep

Date and Time: 5-3-14Performed by: MATTHEW MORTENSEN + ANNA GARVEY

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

# Gel Electrophoresis

Page 1467

Name: ANNA GARVEY + MATT MOETZGEN

Date: 5-3-14

Time: 7:45pm

% Agarose: 1.0

Lane Reagents

1

2

3

4

5

6

7

8

N<sub>2</sub>

N<sub>1</sub>

AB

Ladder

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

L:

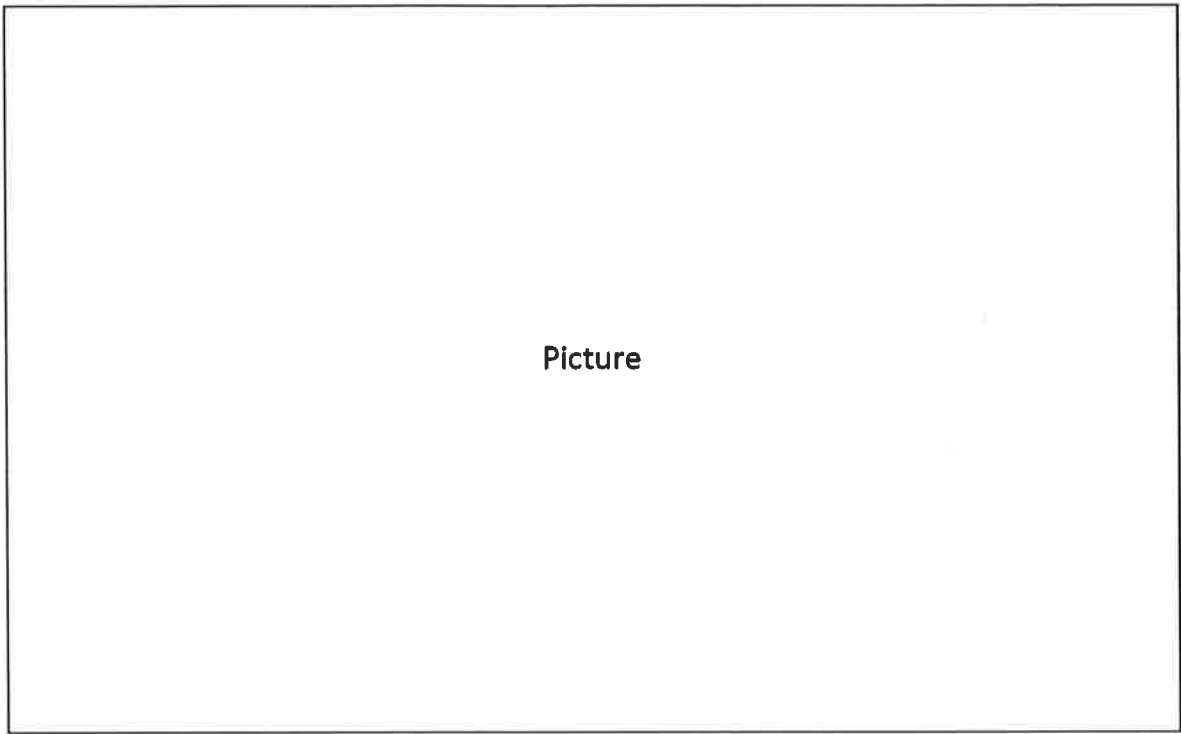
AB: PCR Product AB (2.5μL) + .5LD (p.140)

N<sub>1</sub>: PCR Product N<sub>1</sub> (2.5μL) + .5LD (p.140)

N<sub>2</sub>: PCR Product N<sub>2</sub> (2.5μL) + .5LD (p.140)

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Picture

Results

|            |
|------------|
| NEXT STEP: |
|------------|



## Zymo Mini Prep

Name(s): ANNA GARVEY + MATT MOERTENSENDate and Time: 5-4-14 2:05amCheck off as you  
complete the steps**Procedure:**Bacteria used: K215010, K215104, J04450

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☒
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☒
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: <Part> AG 421 5-4-14Location of product: -20 Plasmid

Deviations from Procedure and other Notes:



NEXT STEP:

Continued on back? Yes ☐; No ☒

## Gel Electrophoresis

Name: David Schoel  
Date: 5/4/14  
Time: 2:30

Picture

Results

NEXT STEP:

# Gel Electrophoresis

Name: Panel Schmitt

Date: 5/4/14

Time: 2:30

% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

|         |          |
|---------|----------|
|         |          |
|         |          |
|         |          |
|         |          |
| K215104 | miniprep |
| K215010 | miniprep |
| 304450  | miniprep |
| Ladder  |          |

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

- 0.5g agarose
- 50ml 1X TAE Buffer
- 2.5ml gel red
- 2ml loading dye for each 5ml sample
- 5ml ladder

Date and Time: \_\_\_\_\_ Performed by: Daniel Schaefer**Qubit DNA Quantification****Master Mix:**

1. Find out how many samples will need to be analyzed 3
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1100
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 5.5 ml
4. Subtract #3 from #2 1099.5 ml
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA ~~BR~~ Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA ~~BR~~ Reagent Br
8. Vortex lightly to mix. Br

|  |  |
|--|--|
| $\begin{array}{r} 1 \\ 700 \\ 3.5 \\ \hline 696.5 \end{array}$ | $\begin{array}{r} 3 \\ 700 \\ 3.5 \end{array}$ |
|--|--|

**Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

**Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

# Results

| Sample Name                        | Page | Concentration                     |
|------------------------------------|------|-----------------------------------|
| <del>h215010</del> nmlpref Johnson |      | <del>0.0181 mg/mL</del> 3.6 mg/mL |
| h215010 nmlpref                    |      | 0.0837 mg/mL 16.5 mg/mL           |
| <del>h215010</del> nmlpref h215104 |      | 0.307 mg/mL 61.4 mg/mL            |
|                                    |      |                                   |
|                                    |      |                                   |
|                                    |      |                                   |
| nmlpref Johnson                    |      | 0.305 mg/mL 61.0 mg/mL            |
| " h215010                          |      | 0.308 mg/mL 61.6 mg/mL            |
| " h215104                          |      | 2.02 mg/mL 41.7 mg/mL             |
|                                    |      |                                   |
|                                    |      |                                   |
|                                    |      |                                   |
|                                    |      |                                   |
|                                    |      |                                   |
|                                    |      |                                   |

1st time

2nd time

0.0205 mg/mL  
4.11 mg/mL  
calculated sample concentration

$$(e.g.) \frac{61 \text{ mg}}{\text{mL}} = \frac{0.061 \text{ mg}}{\text{mL}} \times \frac{1 \text{ mL}}{0.061 \text{ mg}} = \frac{16.39 \text{ mL}}{\text{mg}}$$

$$(e.g.) \frac{61.6 \text{ mg}}{\text{mL}} = \frac{0.0616 \text{ mg}}{\text{mL}} \times \frac{1 \text{ mL}}{0.0616 \text{ mg}} =$$

Johnson [e.g.] a)  $\frac{61 \text{ mg}}{\text{mL}} \rightarrow \frac{0.061 \text{ mg}}{\text{mL}}$  b) inverse  $\frac{1 \text{ mL}}{0.061 \text{ mg}} = 16.39 \frac{\text{mL}}{\text{mg}}$

h215010 [e.g.] a)  $\frac{61.6 \text{ mg}}{\text{mL}} \rightarrow \frac{0.0616 \text{ mg}}{\text{mL}}$  b) inverse  $\frac{1 \text{ mL}}{0.0616 \text{ mg}} = 16.23 \frac{\text{mL}}{\text{mg}}$

h215104 [e.g.] a)  $\frac{41.7 \text{ mg}}{\text{mL}} \rightarrow \frac{0.417 \text{ mg}}{\text{mL}}$  b) inverse  $\frac{1 \text{ mL}}{0.417 \text{ mg}} = 2.40 \frac{\text{mL}}{\text{mg}}$

Date and Time: 5/4/14 Performed by: Annelschroeder**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB Buffer 2.1
- o DNA 504450, K215010, K215104
- o Enzyme Spe I, EcoRI

| MATERIALS | TYPE           | AMOUNT USED    |
|-----------|----------------|----------------|
| Buffer    | NEB 2.1        | 2.5 ml         |
| DNA       | 504450         | 16.39 ml/mg    |
| Enzyme(s) | Spe I<br>EcoRI | 1.5 of each ml |
| Water     | DEPC           | 3.11 ml        |

Total Reaction Volume 25 mL

Construct a table with this format for each reaction that you do today.

| Materials | Type           | Amt. used      |
|-----------|----------------|----------------|
| Buffer    | NEB 2.1        | 2.5 ml         |
| DNA       | K215010        | 16.23 ml/mg    |
| Enzymes   | Spe I<br>EcoRI | 1.5 of each ml |
| Water     | DEPC           | 3.27 ml        |

| Materials | Type           | Amt. used      |
|-----------|----------------|----------------|
| Buffer    | NEB 2.1        | 2.5 ml         |
| DNA       | K215104        | 2.40 ml/mg     |
| Enzymes   | Spe I<br>EcoRI | 1.5 ml of each |
| Water     | DEPC           | 17.1 ml        |

5/14/14 Note

\* as I was transferring the enzymes into one of these on 5/14/14, I forgot to jot this down, I cracked the microcentrifuge tube I was holding.

- I quickly transferred the material to a new tube before sample leaked out, but if something goes wrong that could be the cause

Start Time of Incubation: 5:00

End Time of Incubation: ~~5:00~~ 6:00

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

65°C [20min]

Where did you store your finished product and what did you label it?

In Plasmid box; labeled (RD) ~~RD~~ 5/14/14 & the name of plasmid

NEXT STEP:

## Zymo Mini Prep

Name(s): Matthew MortensonDate and Time: 5-4-14 2:15Check off as you  
complete the steps**Procedure:**Bacteria used: K873001 (4hr), K25104 (Amp), K25001 (Amp) p145

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☐
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☐
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☐
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zippy Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 30 $\mu$ L Zippy Elution Buffer to column..... ☐
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: <Part> <Resistance> 5-4-14 p152

Location of product: \_\_\_\_\_

Deviations from Procedure and other Notes: Half used QIAgen Neutralization Buffer  
(Labeled w/ a Q)

NEXT STEP:

Continued on back? Yes ☐; No ☐



Date and Time: 5/4/14 4:45Performed by: Matthew Markson

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 6
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ~~\_\_\_\_\_~~
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 6.5  $\mu$ L
- Subtract #3 from #2 127.5  $\mu$ L
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

## PCR

Date and Time: 5-4-2014 4:45pm Performed by: ANNA GARVEY

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer MidSci Bullseye 2xPCR Master Mix 12.5ul

dNTPs    MM

Forward Primer Safety Team PCR Primer A C

Reverse Primer Safety Team PCR Primer B D

Template DNA K314200

Taq DNA Polymerase MM

Nuclease-free water Dep C

### Procedure:

| Symbol on lid    | Content in PCR tube  |
|------------------|--|
| <del>PC</del> CD | 12.5 $\mu$ L MM + 12.5 $\mu$ L Primer A + 12.5 $\mu$ L Primer B + 5 $\mu$ L + 5 $\mu$ L DePC |
| N <sub>1</sub>   | No plasmid $\rightarrow$ 10 $\mu$ L DePC   |
| N <sub>2</sub>   | No Primers $\rightarrow$ 7.5 $\mu$ L DePC  |
|                  |  |
|                  |  |
|                  |  |

### Temperature Settings

|              |                    |
|--------------|--------------------|
| Denaturation | 95°C               |
| Annealing    | <del>55</del> 50°C |
| Extension    | 72°C               |
| Final        | 4°C                |

Number of cycles: 34

AG 5-4-14 Time of completion: \_\_\_\_\_

Label on product(s): K314 200 Seeing PCR Product Location of product(s) PCR Box

End notes/comments:

**NEXT STEP:**

# SEWing PCR

Continued on back? Yes ☐; No ☐

Date and Time: 5/4/14 6:30 am Performed by: Matthew Murtanen**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- Buffer \_\_\_\_\_
- DNA \_\_\_\_\_
- Enzyme \_\_\_\_\_

| MATERIALS | #1<br>TYPE      | #2              | AMOUNT<br>USED #1 #2 |           |
|-----------|-----------------|-----------------|----------------------|-----------|
| Buffer    | NEBuffer<br>2.1 | NEBuffer<br>2.1 | 2.5<br>2.5 $\mu$ L   | 5 $\mu$ L |
| DNA       | K215104<br>p152 | K215001<br>p152 | 3.61                 | 25.9      |
| Enzyme(s) | EcoRI<br>SpeI   | EcoRI<br>XbaI   | 1 $\mu$ L            | 1 $\mu$ L |
| Water     | DEPC            |                 | 16.89                | 17.1      |

Total Reaction Volume 25  $\mu$ L / 50

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: <sup>5</sup> 6:50

End Time of Incubation: 6:50

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

Heat Inactivation : 65°C for 20 min

Where did you store your finished product and what did you label it?

NEXT STEP:

# Gel Electrophoresis

Page 154

Name: ANNA GARVEY

Date: 5-4-14

Time: 7:15

% Agarose: 1%

Lane Reagents

1

2

3

4

5

6

7

8

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

2<sup>+</sup>

2<sup>-</sup>

9

1

Reagents used:

CD: Product CD (2.5mL) + 0.5LD p. 154

N<sub>1</sub>: Product N<sub>1</sub> (2.5mL) + 0.5LD p. 154

N<sub>2</sub>: Product N<sub>2</sub> (2.5mL) + 0.5LD p. 154

L: 5mL Ladder

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

SEWing PCR

## Gel Electrophoresis

Name: Matthew MortensenDate: 5/4/14Time: 7pm% Agarose: 1

Lane Reagents

1

2

3

4

5

6

7

8

L

P<sub>1</sub>D<sub>1</sub>D<sub>4</sub>P<sub>4</sub>

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

L = 1kb NEB DNA ladder 1uL

P<sub>1</sub> = K215001 Plasmid (p152) 2uLD<sub>1</sub> = K215001 Plasmid Digested (p154) 2.5uLD<sub>4</sub> = K215104 Plasmid Digested (p154) 12.5uLP<sub>4</sub> = K215104 Plasmid (p152) 2uL

Start: 7:45

End: 8:50



## Gel Electrophoresis

Name: \_\_\_\_\_

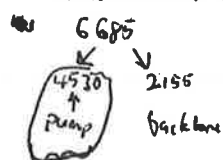
Date: \_\_\_\_\_

Time: \_\_\_\_\_

Picture

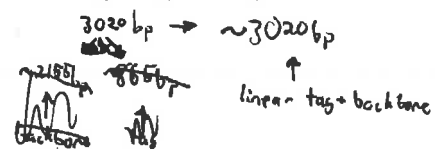
### Results

215104 should have been cut



Put into a 1.037g Microcentrifuge tube  
.387g

215001 should have been cut



.301g Put into a 1.047g Microcentrifuge tube

NEXT STEP: Gel Extraction + Ligation

# Gel Electrophoresis

Page 158

Name: Daniel Schaefer

Date: 5/5/14

Time: 2:10

% Agarose: 1

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

|   |                    |                       |
|---|--------------------|-----------------------|
| 1 | —                  |                       |
| 2 | K215104 (miniprep) |                       |
| 3 | K215104 (R.D.)     |                       |
| 4 | K215104 (miniprep) | → went into gel weird |
| 5 | K215104 (R.D.)     |                       |
| 6 | Don450 (miniprep)  | → went into gel weird |
| 7 | Don450 (R.D.)      |                       |
| 8 | Ladder             |                       |

Reminders:

Add Gel Green

Add Loading Dye

Run Red

} must leaked out of wells, possibly too much buffer or should have added more loading dye to help it sink to the bottom; this is

ok since we are testing R.D. mainly w/ this gel

Reagents used:

0.5g agarose

50mL TAE 1 Buffer

2.5mL gel red

2-lag ladder — 5 ml

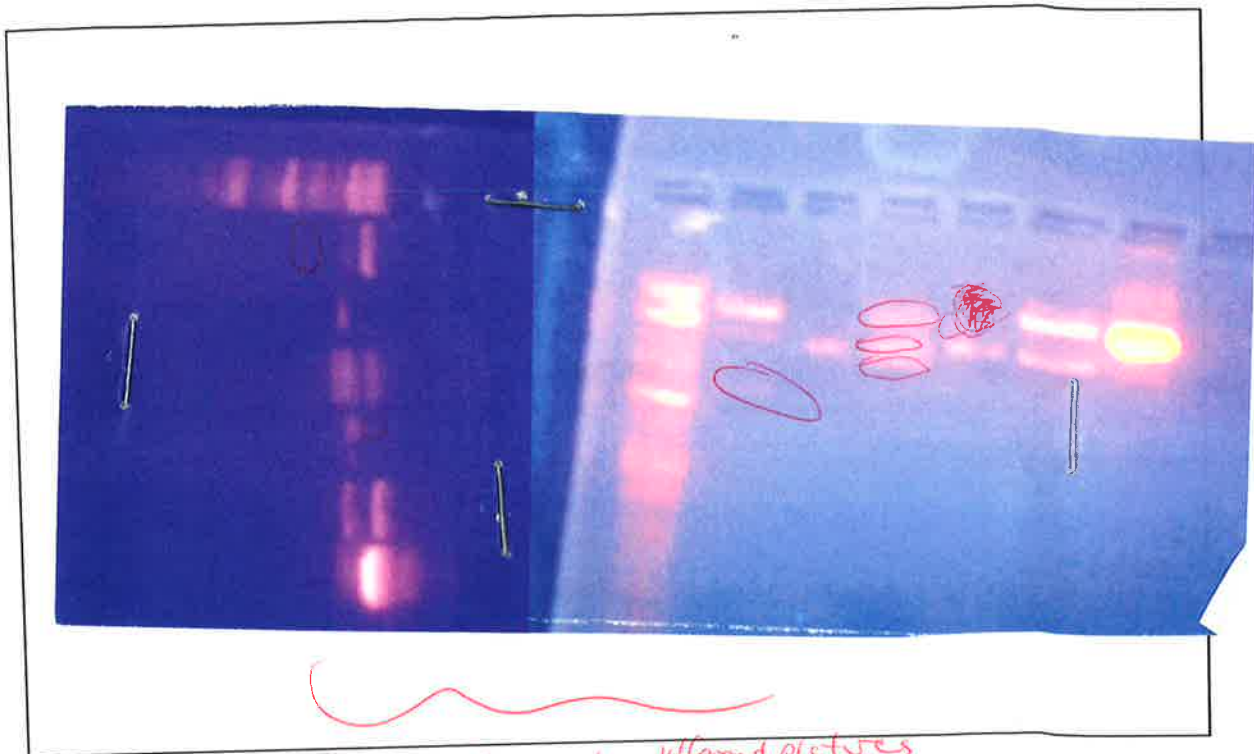
5ml DNA sample + 1ml loading dye

# Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_



Results

same gel, different pictures

NEXT STEP:

SJC  
SEWING

## PCR

Date and Time: 5-5-14 Performed by: ANNA GARVEY

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer MidSci Bullseye 2xPCR MasterMixdNTPs MMForward Primer K315200 (A primer in <sup>AD</sup> MM)Reverse Primer K875001 (D primer in <sup>AD</sup> MM)Template DNA XTaq DNA Polymerase MMNuclease-free water DepC

12.5

5ul

5ul

2.5ul

Procedure:

| Symbol on lid | Content in PCR tube   |
|---------------|---|
| SEW           | 5ul K315200 + 5ul K875001 + 2.5ul DepC + 12.5ul MM                    |
| <del>AD</del> | 5ul K315200 + 5ul K875001 + 12.5ul MM + 1.25 A primer + 1.25 D primer |
| <del>AD</del> | <del>5.5ul MM + 2.5ul DepC</del>                                      |
|               |   |
|               |   |
|               |   |
|               |   |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 60°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) \_\_\_\_\_

End notes/comments:

NEXT STEP:

Run Gel

Continued on back? Yes ☐ ; No ☐

Date and Time: 5-5-14 4pm Performed by: Matthew Martensen

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |              |              |  |  |  |  |  |  |  |
|-------------------|--------------|--------------|--|--|--|--|--|--|--|
| Sample            | K215001 p157 | K215104 p157 |  |  |  |  |  |  |  |
| Tube + Gel weight |              |              |  |  |  |  |  |  |  |
| Empty tube weight |              |              |  |  |  |  |  |  |  |
| Gel Weight        | .301g        | .387g        |  |  |  |  |  |  |  |
| Buffer QG         | 903uL        | 1161uL       |  |  |  |  |  |  |  |
| Isopropanol       | 301uL        | 387          |  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100μL per 100mg; so 3 volumes is 300μL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800μL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750μL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30μL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

K215104 didn't work see p 162

Label on Product: Part 7 Res Dig Purification p160

Location of Product: Primer Box

Next Step: Ligate

Date and Time: 5-5-14 10:30pm Performed by: Anny Gavey Matthew Montensen**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- Buffer NEB 2.1
- DNA \_\_\_\_\_
- Enzyme \_\_\_\_\_

| MATERIALS | TYPE           | AMOUNT USED                         |
|-----------|----------------|-------------------------------------|
| ✓ Buffer  | NEB 2.1        | <del>5</del> <u>5</u> $\mu\text{L}$ |
| DNA       | K215104 (p148) | 2.41 $\mu\text{L}$                  |
|           | J04450 (p148)  | 5.92 $\mu\text{L}$                  |
| Enzyme(s) | EcoRI + SpeI   | 1 $\mu\text{L}$ each                |
| ✓ Water   | DEPC           | K = 15.99   J = 12.08               |

Total Reaction Volume 25  $\mu\text{L}$ 

Construct a table with this format for each reaction that you do today.

K 215104 (#1) 415  $\mu\text{g}/\text{mL}$   
 J04450 (#2) 169  $\mu\text{g}/\text{mL}$

$$\frac{415 \mu\text{g}}{1 \text{ mL}} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} \times \quad = \frac{1 \mu\text{g}}{\mu\text{L}}$$

$$\frac{415 \mu\text{g}}{100 \mu\text{L}} = \frac{1 \mu\text{g}}{x \text{ mL}}$$

$$\frac{0.415 \mu\text{g}}{1 \mu\text{L}} = \frac{1 \mu\text{g}}{x}$$

$$x = 2.41 \mu\text{L}$$

$$\frac{169 \mu\text{g}}{\text{mL}} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} = \frac{0.169 \mu\text{g}}{\mu\text{L}}$$

$$\frac{0.169 \mu\text{g}}{1 \mu\text{L}} = \frac{1 \mu\text{g}}{x}$$

$$= 5.92 \mu\text{L}$$

Start Time of Incubation: 10:50pm

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

Heat Inactivation at 65°C for 20min

Where did you store your finished product and what did you label it?

<part> AG 5-5-14 p168

NEXT STEP:

Primer

~~Plasmid~~ Box (PCR section)

run gel/tubit

Date and Time: 5-5-14 5:05pm Performed by: ANNA GARVEY + MATT MORTENSEN

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

Date and Time: 5-5-14 9:20 pm Performed by: Matt Morlensen

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed \_\_\_\_
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 \_\_\_\_  $1\mu\text{L} + 199\mu\text{L}$   
Reagent Buffer
- Subtract #3 from #2 \_\_\_\_
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

# Gel Electrophoresis

Page 1184

Name: Matthew Mortensen

Date: 5/5/14

Time: 9:30

% Agarose: 1

Lane Reagents

|   |                 |                    |
|---|-----------------|--------------------|
| 1 | L               | 1uL                |
| 2 | P <sub>04</sub> | 2uL p152           |
| 3 | D <sub>04</sub> | 20uL p154 ← (p155) |
| 4 | AB              | 3uL p 140          |
| 5 | AD              | 3uL p159           |
| 6 | SEW             | 3uL p 159          |
| 7 | CD              | 3uL p154           |
| 8 |                 |                    |

Reminders:

Add Gel Green

Add Loading Dye

Run Red

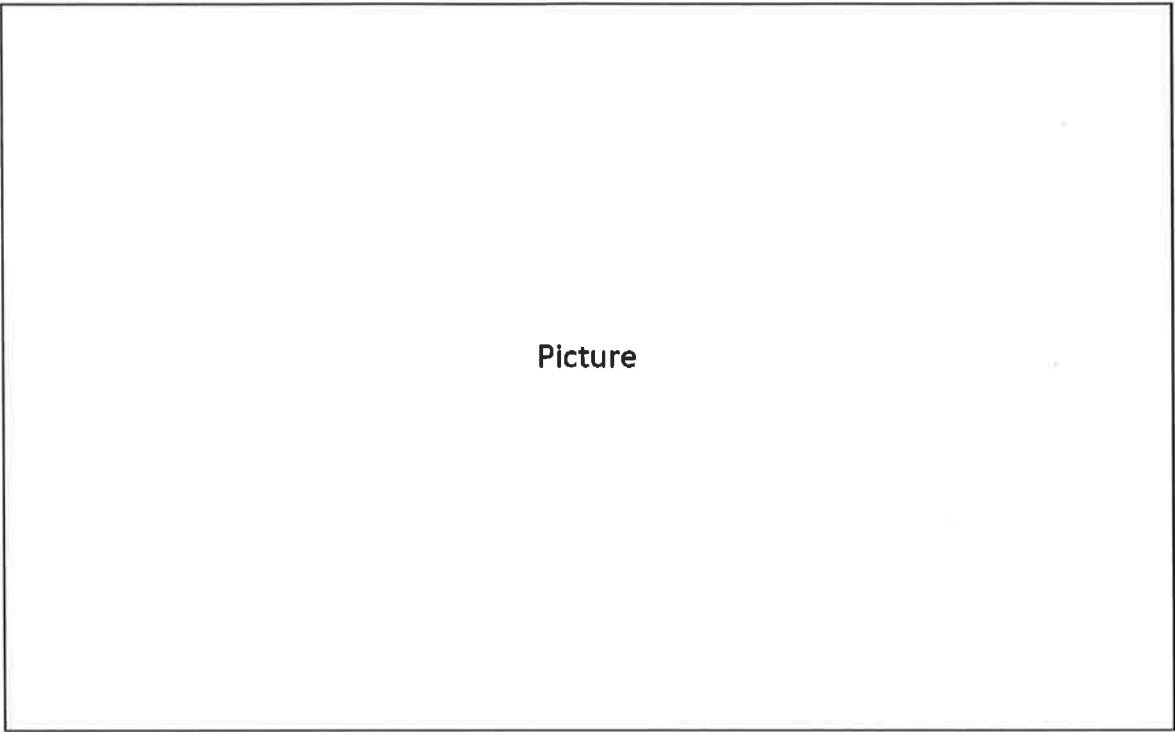
Started @ 10:17 pm

Reagents used:

36g Gel Fragment was put into 1.032g Turbo  
Pump

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Picture

Results

NEXT STEP:

Date and Time: 5/6/14 2:15 pm Performed by: JOSHUA RICE & MATTY MORTENSEN

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |              |  |  |  |  |  |  |  |  |
|-------------------|--------------|--|--|--|--|--|--|--|--|
| Sample            |              |  |  |  |  |  |  |  |  |
| Tube + Gel weight | 1.395g       |  |  |  |  |  |  |  |  |
| Empty tube weight | 1.032g       |  |  |  |  |  |  |  |  |
| Gel Weight        | .363 g       |  |  |  |  |  |  |  |  |
| Buffer QG         | 1108 $\mu$ L |  |  |  |  |  |  |  |  |
| Isopropanol       | 363 $\mu$ L  |  |  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: ON TOP: 166 ON SIDE K215404 Pump 5/6 R

Location of Product: iGCM Primers Box

Next Step: Ligation

Date and Time: \_\_\_\_\_  
Protocol: R Qubit  
Reagents: Quant-iT RNA BR kit

Lab Technicians(s) involved: Matt Mortensen  
Tashia Piche

## Procedure (with applicable notes):

Rn = Qubit of 1215104 (p166) Gel Extract

## Results:

7.93 uL/mL

Location of product: \_\_\_\_\_  
Label on product: \_\_\_\_\_  
End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐

# Gel Electrophoresis

Page 168

Name: Daniel Schaefer, Josh Kersnowsky

Date: 5/6/19 Zach Birner

Time: 5:30-6:40

% Agarose: 1

Lane Reagents

|   |                |
|---|----------------|
| 1 |                |
| 2 |                |
| 3 |                |
| 4 |                |
| 5 | K215010 (R.D.) |
| 6 | K215101 (R.D.) |
| 7 | Johnson (R.D.) |
| 8 | Ladder         |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

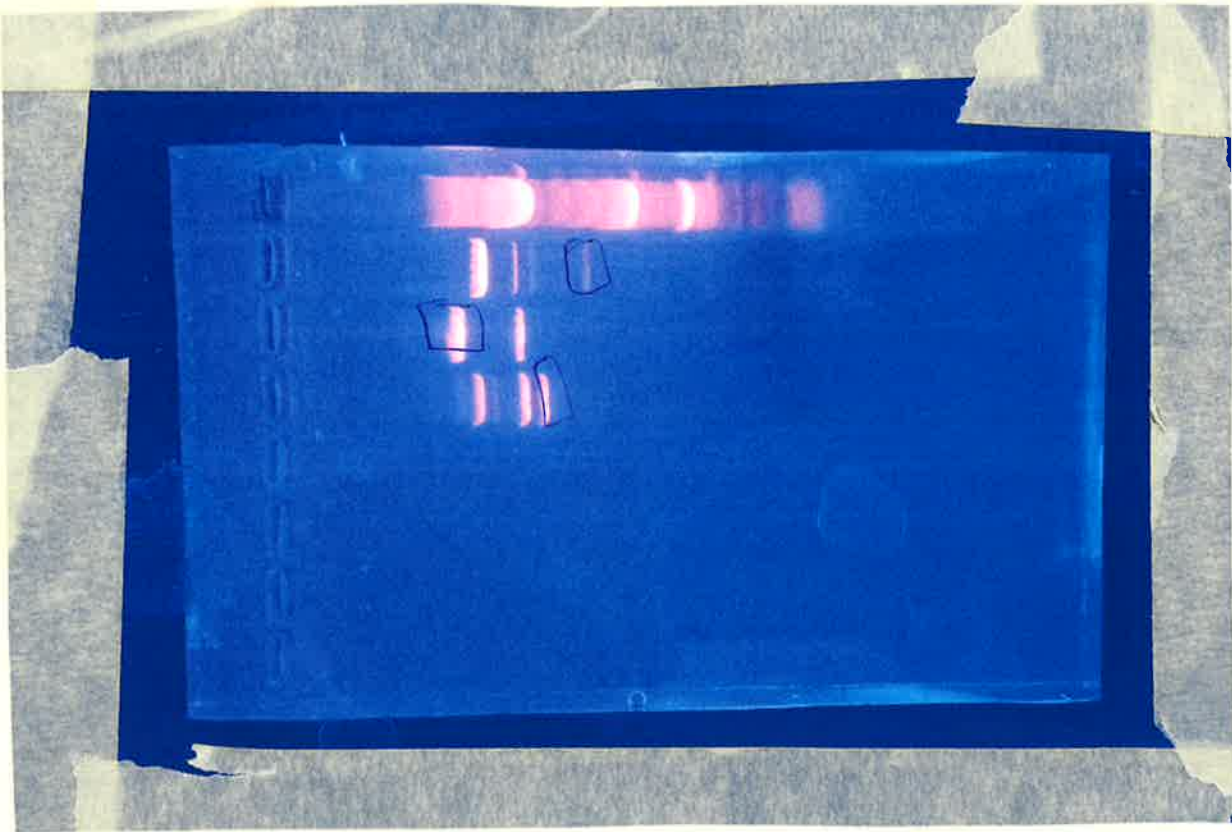
1.5g Agarose  
50ml TAE XI Buffer  
2.5ml gel red

5ml sample DNA  
2ml loading dye  
5ml 2log ladder



# Gel Electrophoresis

Name: Josh Klesner, Daniel Schuch, Zach Birner  
 Date: 5/16/14  
 Time: \_\_\_\_\_



(R.E.)  
 Controls: - each restriction enzyme separately  
 - no RD/plasmid

(6x)  
 Ladder      NO R.E.      + plasmid      + plasmid      + plasmid  
                  (-)      + SpeI      - SpeI      + SpeI  
                  (+ plasmid)      - EcoRI      + EcoRI      + EcoRI  
                  / Min Amount (plas) For Visibility

Continue For each Restriction Digest / plasmid

Lot of Insert, small amount of vector

NEXT STEP:

Gel extraction

Ligation  
 NO I      NO Lig      no vector      1ul Vector      1ul Vector  
 NO Lig      —      —      1ul Insert      5ul Insert      10ul Insert  
 ctrls      —      —      1:1      1:5      1:10

Date and Time: 5/6/14 7:00 Performed by: Danel Schreder, Josh Leverage

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |                                |                                 |                                 |  |  |  |  |  |  |
|-------------------|--------------------------------|---------------------------------|---------------------------------|--|--|--|--|--|--|
| Sample            | Johnson                        | KZ15104                         | KZ15120                         |  |  |  |  |  |  |
| Tube + Gel weight | 1.32g<br><small>1.32mg</small> | 1.248<br><small>1.248mg</small> | 1.211<br><small>1.211mg</small> |  |  |  |  |  |  |
| Empty tube weight | 1.04g<br><small>1.04mg</small> | 1.03g<br><small>1.03mg</small>  | 1.03g<br><small>1.03mg</small>  |  |  |  |  |  |  |
| Gel Weight        | 280mg                          | 218mg                           | 181mg                           |  |  |  |  |  |  |
| Buffer QG         | 840μL                          | 654μL                           | 543μL                           |  |  |  |  |  |  |
| Isopropanol       | 280μL                          | 218μL                           | 181μL                           |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100μL per 100mg; so 3 volumes is 300μL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800μL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750μL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30μL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: Gel extraction; KZ15104, KZ15109, Johnson; 5/6/14

Location of Product: Plasmid box

Next Step: Ligation

Date and Time: \_\_\_\_\_  
 Protocol: Ligation w/ T4 DNA Ligase (NEB)  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: ANNA GARNEY  
RYAN GEORGE  
MATT MORTENSEN

Procedure (with applicable notes):

| 2 uL 10X T4 DNA Ligase Buffer   |                    |                       |                 |                                       |
|---------------------------------|--------------------|-----------------------|-----------------|---------------------------------------|
| 1 uL DNA Ligase                 | 1 uL T4 DNA Ligase | 1 uL T4 DNA Ligase    | —               |                                       |
| K215104 "Pump" (p160)           | K215104 (p160)     | —                     | K21504 (p160)   | K215104: 16.31 uL<br>K215001: 1.12 uL |
| K215001 "Tag + Backbone" (p160) | —                  | K215001 (p160)        | K215001 (p160)  |                                       |
| Dep C: 11.57 uL                 | Dep C Water: 12.49 | Dep C Water: 17.88 uL | Dep C: 12.57 uL |                                       |

Results:

Location of product: iGEM Primer Box

Label on product: K215004 + K215001 <L-N3> AG 5-8-14

End Notes/Comments: Page # may look like 172, I wrote over the 2 with a 0

NEXT STEP:

Electroporation

Continued on back? Yes ☐; No ☒

Date and Time: 5/7/11 9:00pm

Protocol: Qubit

Reagents: Quant-it dsDNA BR Buffer  
" " " " " Reagent

Quant-it dsDNA BR standards #1 + #2

Lab Technicians(s) involved: Ryan George  
Sash Leverette  
Anna Garvey

pg. 159 - Sequencing PCR product AD and SEW

Procedure (with applicable notes):

Mix 1000  $\mu$ L working solution in a microfuge tube

- 5  $\mu$ L Quant-it Reagent
- 995  $\mu$ L Quant-it Buffer

Make Standards

#1 - 10  $\mu$ L standard 1 with 190  $\mu$ L working solution

#2 - " " 2 " " " "

Make Samples

AD - 1  $\mu$ L product AD w/ 199  $\mu$ L working solutionSEW - 1  $\mu$ L product SEW w/ 199  $\mu$ L working solution

- Run Qubit

Results:

AD - 197  $\mu$ g/mLSEW - ~~520  $\mu$ g/mL~~ 117  $\mu$ g/mLSuccess

Location of product: iGEM Primers Box

Label on product: 159, same tubes as we started with

End Notes/Comments: We're good!

NEXT STEP:

Continued on back? Yes ☐; No ☒

Name(s): Anna Carvey  
Matt Mortensen  
Josh Lawrence

## Electroporation

Page: 172

Reagents: LB

### Cell Preparation

Competent cells thawed? Yes ☒ No ☐

Amount? 50 mL

### Electroporation

Electroporation cuvette chilled? Yes ☒ No ☐

Amount of SOC added to culture tubes: 1 mL

DNA placed in m/f tubes? Yes ☒ No ☐

Placed on ice? Yes ☒ No ☐

Amount of DNA mixed with competent cells: 2 mL

Voltage used for electroporation: EC01

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No ☐

Amount added: 1 mL

Cells shaken at 37°C? Yes ☒ No ☐

Start time: 9:00 End time: 9:10

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes ☒ No ☐

Cells spun in centrifuge (10-15s)? Yes ☒ No ☐

s/n decanted off? Yes ☒ No ☐

Cells resuspended? Yes ☒ No ☐

Amount of solution: 100 mL

Dilutions performed:

10<sup>-1</sup> Yes ☒ No ☐

10<sup>-2</sup> Yes ☒ No ☐

10<sup>-3</sup> Yes ☒ No ☐

10<sup>-4</sup> Yes ☒ No ☐

10<sup>-5</sup> Yes ☐ No ☒

10<sup>-6</sup> Yes ☐ No ☒

10<sup>-7</sup> Yes ☐ No ☒

10<sup>-8</sup> Yes ☐ No ☒

10<sup>-9</sup> Yes ☐ No ☒

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: <L1 - L8> <Dilution Factor> 5-8-14 JL

Location of products: Warm Room

Next steps: See what grew

Signature: Anna Carvey

From: <http://www.oardc.ohio-state.edu/stookingerlab/Protocols/Electroporation.pdf>

Date and Time: 5/8/14

Performed by: Jacob Soder

**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB 2.1
- o DNA SEW, AD, K875001 (not a)
- o Enzyme Xba I, Pts I

$$\frac{SEW\ 1\ mg}{117\ \mu g} \times \frac{1000\ \mu L}{1\ mL} = 8.547\ mL\ SEW$$

$$\frac{K875001\ 1\ mg}{136\ \mu g} \times \frac{1000\ \mu L}{1\ mL} = 7.353\ mL\ K875001$$

$$\frac{AD\ 1\ mg}{197\ \mu g} \times \frac{1000\ \mu L}{1\ mL} = 5.076\ mL\ AD$$

SEW and AD from pg. 171  
K875001 from pg. 153

| MATERIALS | TYPE        | AMOUNT USED |
|-----------|-------------|-------------|
| Buffer    | NEB 2.1(10) | 2.5 mL      |
| DNA       | K875001     | 7.35 mL     |
| Enzyme(s) | Xba I       | 1 mL        |
|           | Pts I       | 1 mL        |
| Water     | DEPC        | 13.15 mL    |

Label K PCR tube

- control

NEB 2.1 2.5 mL  
K875001 7.35 mL  
DPL 13.15 mL  
no enzymes

Label

- K PCR tube

Total Reaction Volume 25 mL

Construct a table with this format for each reaction that you do today.

|        |                        |           |         |          |
|--------|------------------------|-----------|---------|----------|
| Buffer | NEB <sup>(b)</sup> 2.1 | 2.5 mL    | NEB 2.1 | 2.5 mL   |
| DNA    | AD                     | 5.076 mL  | SEW     | 8.547 mL |
| Enzyme | Xba I                  | 1 mL      | Xba I   | 1 mL     |
|        | Pts I                  | 1 mL      | Pts I   | 1 mL     |
| Water  | DEPC                   | 15.424 mL | DEPC    | 11.95 mL |

Label AD PCR tube

Label S

Label S PCR tube

|            |           |            |          |
|------------|-----------|------------|----------|
| NEB 2.1    | 2.5 mL    | NEB 2.1    | 2.5 mL   |
| AD         | 5.076 mL  | SEW        | 8.547 mL |
| DEPC       | 17.424 mL | DEPC       | 13.95 mL |
| no enzymes |           | no enzymes |          |

Label -AD PCR tube

Label -S PCR tube

NOTE: There was not enough SEW product to conduct a negative control so no - control was done

- controls

Start Time of Incubation: 10:23 pm

End Time of Incubation: 11:44 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

In plasmid box

---

Label on products

|                                |                    |
|--------------------------------|--------------------|
| 1525001                        | <del>173</del> 173 |
| sewing product 1D              | 173                |
| sewing product sew             | 173                |
| + 1525001 no enzyme            | 173                |
| sewing product 1D<br>no enzyme | 173                |

*1525001*

NEXT STEP:

ligate

Name(s): Jacob Jadet  
Anne Garvey

Date and Time: 11:00 5/8/14

### Liquid Culture

Amount of LB used: 5 mL

Part Used: missile 1217 from Genomic DNA box

Antibiotic Used: NONE

Amount of Antibiotic Used: \_\_\_\_\_

Concentration of Antibiotic: \_\_\_\_\_

I did 13 cultures

Label on Product: Ecoli Missle 1917

Location of Product: Warm room

Next Step: \_\_\_\_\_



Name(s): JOSIAH RICKEDate and Time: 5/9/14 4:50 pmLiquid CultureAmount of LB used: 5 mLPart Used: Pump + Tag (FROM PAGE 172)Antibiotic Used: AmpAmount of Antibiotic Used: 10 mLConcentration of Antibiotic: 100 µg/mL

L1 Product

Amount of LB used 5 mLPart Used Pump + Tag (FROM PG 172)Antibiotic Used AmpAmount Antibiotic Used 10 mLConcentration of Antibiotic 100 µg/mL

L3 Product

Label on Product: Product of L1 or L3 Pump + Tag 5/9 RLocation of Product: Warm RoomNext Step: ~~Mini Prep~~ (But more importantly check for growth)  
Glycerol stock

Date and Time: 6/9/14 5:45pm  
 Protocol: G-bit  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: JOSHUA RICE  
MM  
Anna G

Procedure (with applicable notes):

Using the digest of K875001 - ~~27~~  
 " " of SEW PCR product  
 " " of SEW PCR + AD product

① } Standards - get 190  $\mu$ L of working solution  
 ② }  
 K - K875001 - get 199  $\mu$ L " " "  
 S - SEW PCR product " " "  
 AD - SEW PCR + AD prod " " "

Results:

① - 37.0  $\mu$ g/mL - 4351.35  
 ⑤ - 34.4  $\mu$ g/mL - 1453.49  
 ⑨ - 30.9  $\mu$ g/mL - 1618.12

} How much necessary for 50 nanograms ~~50~~

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 5-9-14  
 Protocol: T4 DNA Ligase  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: ANNA GARVEY  
 MATTHEW MORTENSON  
 JOSIAH RICE

Procedure (with applicable notes):

| 2 $\mu$ L of 10x T4 DNA Ligase Buffer   |  |   |  |   |  |
|---|--|---|--|---|--|
| Negatives   |  |   |  |   |  |
| Actual Ligation<br>SEW (S)  | Actual Ligation<br>SEW + AD (A)  | (N1)  | (N2)   | (N3)  | (N4)   |
| 1 $\mu$ L DNA Ligase<br>KB75001 Digest:<br>1.35 $\mu$ L<br>SEW PCR Digest:<br>1.45 $\mu$ L<br>DepC:<br>14.2 $\mu$ L | 1 $\mu$ L DNA Ligase<br>KB75001 Digest:<br>1.35 $\mu$ L<br>Sewing PCR + AD<br>digest:<br>1.45 $\mu$ L<br>1.62 $\mu$ L<br>DepC: 14.03 $\mu$ L | 1 $\mu$ L DNA Ligase<br>KB75001 Digest:<br>1.35 $\mu$ L<br>—<br>DepC: 15.65 $\mu$ L | 1 $\mu$ L DNA Ligase<br>—<br>Sewing PCR<br>digest:<br>1.45 $\mu$ L<br>DepC:<br>15.55 $\mu$ L | —<br>KB75001 Digest:<br>1.35 $\mu$ L<br>Sewing PCR<br>Digest<br>1.45 $\mu$ L<br>DepC:<br>15.2 $\mu$ L | 1 $\mu$ L DNA Ligase<br>—<br>Sewing PCR + AD<br>Digest<br>1.62 $\mu$ L<br>DepC:<br>15.38 $\mu$ L |

Results:

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Name(s): ANNA GARVEY **Electroporation**  
MATTHEW MORTENSEN

Page: 177

Reagents: \_\_\_\_\_

### Cell Preparation

Competent cells thawed? Yes ☒ No ☐ (*E. coli* Nissle 1917) Amount? 50  $\mu$ L

### Electroporation

Electroporation cuvette chilled? Yes ☒ No ☐

Amount of <sup>LB</sup> ~~SOC~~ added to culture tubes: 5 mL

DNA placed in m/f tubes? Yes ☒ No ☐

Placed on ice? Yes ☒ No ☐

Amount of DNA mixed with competent cells: 2  $\mu$ L

Voltage used for electroporation: 200 V

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

<sup>LB</sup> ~~SOC~~ immediately added after electroporation? Yes ☒ No ☐

Amount added: 1 mL

Cells shaken at 37°C? Yes ☒ No ☐

Start time: \_\_\_\_\_ End time: \_\_\_\_\_

### Serial Dilution - No Serial Dilution was done

Cells transferred to 1 mL m/f tube? Yes ☒ No ☒

Cells spun in centrifuge (10-15s)? Yes ☒ No ☒

s/n decanted off? Yes ☒ No ☒

Cells resuspended? Yes ☒ No ☒

Amount of solution: \_\_\_\_\_

Dilutions performed:

10<sup>-1</sup> Yes ☐ No ☐

10<sup>-2</sup> Yes ☐ No ☐

10<sup>-3</sup> Yes ☐ No ☐

10<sup>-4</sup> Yes ☐ No ☐

10<sup>-5</sup> Yes ☐ No ☐

10<sup>-6</sup> Yes ☐ No ☐

10<sup>-7</sup> Yes ☐ No ☐

10<sup>-8</sup> Yes ☐ No ☐

10<sup>-9</sup> Yes ☐ No ☐

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: <Lysate Product> AG 5-8-14

Location of products: Primer Box

Next steps: Plate

Signature: Anna Garvey

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

\*it seemed from the results that *E. coli* Nissle 1917 is chloramphenicol resistant, its resistance to all three antibiotics are being tested.

Date and Time: 5/10/14  
Protocol: Plating  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: Matthew Mortensen  
Ann Garvey  
\_\_\_\_\_

Procedure (with applicable notes):

Plated 100 $\mu$ L of liquid culture of Ecoli Nissle 1917 on 1 amp, 1 chloro, and 1 kan plate  
in order to check for natural resistances

Results:

no natural resistances - Christa Winslow 5/14/14

Location of product: Warm Room

Label on product: Ecoli Nissle 1917 mwm AG 5/10/14

End Notes/Comments: check in 12-24 hours

NEXT STEP:

Continued on back? Yes ☐; No ☐

## Gel Electrophoresis

Name: Devil Subach, Josh LechnerDate: 5/14/14Time: 1:502:30 - 3:40 PM% Agarose: 1

Lane Reagents

1

2

3

4

5

1215010CRP)

6

1215104 (RD)

7

1215104 (RD)

8

1215104

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

0.5g Agarose + 50ml x1 TAE5ml DNA sample + 1ml dye5ml 2x loading dye

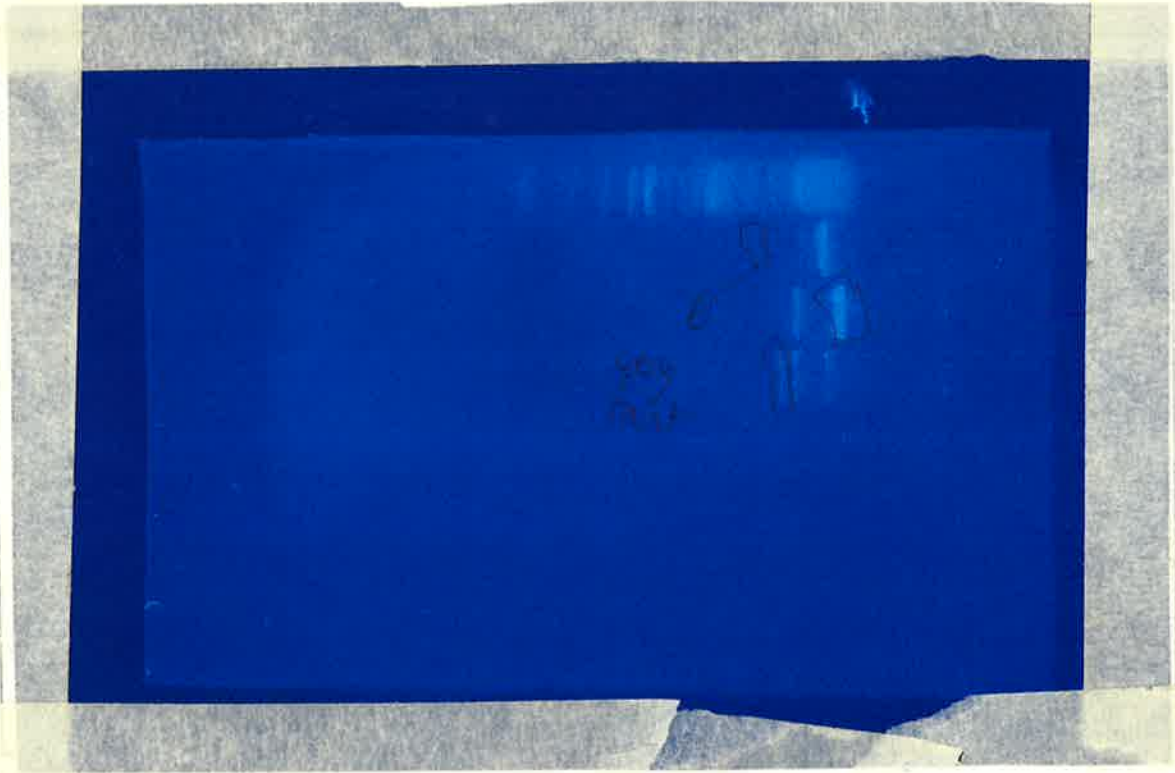
- Notes:
- we are doing this to prepare for another round of gel extraction [we believe we extracted the right DNA last time, but we are making sure with this]
  - we are also doing this because, though we have some <sup>1</sup>extracted DNA from 5/6/14, we are not comfortable with adding <sup>product</sup> into the cells through electroporation yet [without Anna or Matt being present]
  - This is because we extracted poorly the previous time

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_



RESULTS

NEXT STEP:

[in case there is any issue w/ our last extraction from 5/11/14]

Date and Time: 5/14/14, 3:45 PM

Performed by: Daniel Schroeder, Josh Keenane

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |                           |                  |             |  |  |  |  |  |  |
|-------------------|---------------------------|------------------|-------------|--|--|--|--|--|--|
| Sample            | J24450                    | K21804           | K215010     |  |  |  |  |  |  |
| Tube + Gel weight | <del>1.222</del><br>1.114 | 1.131            | 1.114       |  |  |  |  |  |  |
| Empty tube weight | 1.022                     | <del>1.038</del> | 1.033       |  |  |  |  |  |  |
| Gel Weight        | 92 mg                     | 103 mg           | 81 mg       |  |  |  |  |  |  |
| Buffer QG         | 276 $\mu$ L               | 309 $\mu$ L      | 243 $\mu$ L |  |  |  |  |  |  |
| Isopropanol       | 92 $\mu$ L                | 103 $\mu$ L      | 81 $\mu$ L  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: PS-180 CS114/1M 3mL; K215010, K215104; Gel extraction

Location of Product: plus and box

Next Step: <sup>Qubit</sup> quantification; electroporation



Sewing  
PCR

Date and Time: 5/15/14

Performed by: Christa Window, Jacob Sadat

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer Bullseye 2x PCR master mix 12.5 mL  
 dNTPs in M/M  
 Forward Primer K315200 (p.154) (A primer in tube A, 1.25 mL) 5 mL  
 Reverse Primer K875001 (p.144) (B primer in tube A, 1.25 mL) 5 mL  
 Template DNA X  
 Taq DNA Polymerase in M/M  
 Nuclease-free water Dep C in tube B 2.5 mL

25 mL RXN

## Procedure:

There was not enough K875001, or K315200 for tube B, so only same was put in that tube

Flanking primers A, and D were put in tube A because there has been more success when the flanking primers are present

| Symbol on lid | Content in PCR tube   |
|---------------|---|
| A             | 5 mL K315200, 5 mL K875001, 12.5 mL M/M, 1.25 mL A primer, 1.25 mL D primer |
| B             | 5 mL K315200, 5 mL K875001, 12.5 mL M/M, 2.5 mL Dep C                       |
|               |   |
|               |   |
|               |   |
|               |   |
|               |   |

## Temperature Settings used BGLS

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 60°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: 5:30

Label on product(s): PCR product A 181  
PCR product B 181

Location of product(s) PCR box

End notes/comments:

p.154 K315200 are gone (all used up)  
 p.144 K875001

## NEXT STEP:

Run Gel, <sup>electrophoresis</sup> extract, purify

Continued on back? Yes ☐; No ☒

# Gel Electrophoresis

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Name: Christa Winslow + Jacob Jodaf

Date: 5/15/14

Time: 6:45 pm

% Agarose: 1%

Lane Reagents

loading dye and (ladder were found in the biobrick box)

1

2

3

4

5

6

7

8

2.5 mL B (p.181) + .5 mL loading dye

2.5 mL A (p.181) + .5 mL loading dye

Ladder already has loading dye in it  
mL - .1 - 10 kb

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Started gel at 7:09 pm

End gel at

Reagents used:

.54g Agarose LE

50 mL 1X TAE Buffer

2.5 mL gel red

A = Sewing PCR product w/ flanking primers

B = Sewing PCR product w/o flanking primers

gel started running at 7:10 pm at 100 V

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Picture

### Results

successful! Strong band for the lane with "A" and the flanking primers. Band estimated at  $\approx 400$ bp, we were looking for a 562 band. We should double check with another person though.

we can't tell if the band in lane A is the Sewing PCR product or part K314200  
562 bp                      477 bp

### NEXT STEP:

~~Qubit on PCR product A (p184)~~

Do Gel on Sewing products next to K875001 and K314200, if the Sewing product is slightly higher than K314200 then Sewing PCR was probably successful

Date and Time: 5/30/14 Performed by: Daniel School

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 6
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1700  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 8.5  $\mu$ L
4. Subtract #3 from #2 1691.5  $\mu$ L
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

| Sample Name                         | Page | Concentration                    |
|-------------------------------------|------|----------------------------------|
| K215104 (Gel extraction) 5/6/14     |      | 0.0195 mg/mL / 3.90 mg/mL → 12.8 |
| K21610 (u " )                       |      | 0.0163 mg/mL / 3.26 mg/mL → 15.3 |
| 761150 (u " )                       |      | 0.0105 mg/mL / 2.10 mg/mL → 23.8 |
| K215104 (Restriction digest) 5/4/14 |      | 0.0577 mg/mL / 11.5 mg/mL → 4.3  |
| K215010 (u " )                      |      | 0.126 mg/mL / 25.3 mg/mL → 2     |
| 761150 (u " )                       |      | 0.154 mg/mL / 30.9 mg/mL → 1.6   |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |
|                                     |      |                                  |

(e.s.)

↳ for isolation →  $3.90 \frac{\text{mg}}{\text{mL}} \times 1000 = 3900 \frac{\text{ng}}{\text{mL}} \rightarrow \frac{3900}{1000} = 3.9 \rightarrow \frac{50}{3.9} = 12.82$

Date and Time: 5/30/14  
 Protocol: Ligation  
 Reagents:

Lab Technicians(s) involved: Zach Birner  
 Daniel Schreier

Procedure (with applicable notes):

|                      | Digest  |         | Digest  |         | Gel extract                  |                              |
|----------------------|---------|---------|---------|---------|------------------------------|------------------------------|
|                      | K215010 | K21510  | K215104 | K215104 | K215010                      | K215104                      |
| BEPc water           | 10.7 ml | 10.7 ml | 11.1 ml | 11.1 ml | —                            | —                            |
| T4 DNA ligase        | 1 ml    | 1 ml    | 1 ml    | 1 ml    | 1 ml                         | 1 ml                         |
| T4 DNA ligase Buffer | 2 ml    | 2 ml    | 2 ml    | 2 ml    | 2 ml                         | 2 ml                         |
| Vector DNA           | 4.3 ml  | 4.3 ml  | 4.3 ml  | 4.3 ml  | 8.5 ml                       | 8.5 ml                       |
| Inserted DNA         | 2 ml    | 2 ml    | 1.6 ml  | 1.6 ml  | <del>4.25 ml</del><br>8.5 ml | <del>4.25 ml</del><br>8.5 ml |

Results:

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Electroporation

Continued on back? Yes ☐; No ☐

Name(s): DANIEL SCHRODER **Electroporation**  
ANNA GARVEY

Page: 185

**DNA** R.D. k213107, k215010, J04450  
**Reagents:** gel extract k213107, k215010, J04450

### Cell Preparation

Competent cells thawed? Yes ☒ No ☐ Amount? 6 conical tubes

### Electroporation

Electroporation cuvette chilled? Yes ☒ No ☐

Amount of <sup>LB broth</sup> ~~SSC~~ added to culture tubes: 4 ml 50 ml

RD = restriction digest  
GE = gel extraction

DNA placed in m/f tubes? Yes ☒ No ☐

Placed on ice? Yes ☒ No ☐

Amount of DNA mixed with competent cells: 2 ml

Voltage used for electroporation: EC 1

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

<sup>LB broth</sup> ~~SSC~~ immediately added after electroporation? Yes ☒ No ☐

Amount added: 1 ml

Cells shaken at 37°C? Yes ☒ No ☐

Start time: 4:30 End time:       

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes ☒ No ☐

Cells spun in centrifuge (10-15s)? Yes ☒ No ☐

s/n decanted off? Yes ☒ No ☐

Cells resuspended? Yes ☒ No ☐

Amount of solution: 1 ml

Dilutions performed:

10<sup>-1</sup> Yes ☒ No ☐  
10<sup>-2</sup> Yes ☒ No ☐  
10<sup>-3</sup> Yes ☒ No ☐  
10<sup>-4</sup> Yes ☒ No ☐  
10<sup>-5</sup> Yes ☒ No ☐  
10<sup>-6</sup> Yes ☐ No ☒  
10<sup>-7</sup> Yes ☐ No ☒  
10<sup>-8</sup> Yes ☐ No ☒  
10<sup>-9</sup> Yes ☐ No ☒

10<sup>-3</sup> } 15 plates  
10<sup>-4</sup> } for each  
10<sup>-5</sup> }

Volumes: 1 ml  
Volumes:         
Volumes:         
Volumes:         
Volumes:         
Volumes:         
Volumes:         
Volumes:         
Volumes:       

Labels on products: 0°, 0A°, 0B° / 4°, 4A°, 4B°

Location of products: -20, primer box

Next steps: plate / liquid culture

Signature:       

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>



|         |   |    |        |           |           |
|---------|---|----|--------|-----------|-----------|
| K215010 | B | RD | $10^0$ | $10^{-2}$ | $10^{-3}$ |
| K215010 |   | GE | $10^0$ | "         | "         |
| K215010 | A | RD | $10^0$ | "         | "         |
| K215104 | B | RD |        |           |           |
| K215104 |   | GE |        |           |           |
| K215104 | A | RD |        |           |           |

$10^0$   
1ml

100:900  
(-1)

10:990  
(-2)



Date and Time: 5-30-14Lab Technicians(s) involved: ANNA GARVEYProtocol: LigationReagents: T4 DNA Ligase BufferT4 DNA Ligase

Procedure (with applicable notes):

- ✓ 1  $\mu$ L DNA ligase
- ✓ KB75001 Digest: 1.35  $\mu$ L
- ✓ SEWing PCR + AD Digest: 1.62  $\mu$ L
- ✓ DepC: 14.03  $\mu$ L
- ✓ 2  $\mu$ L of 10x T4 DNA Ligase Buffer

Results:

Location of product:

Label on product:

End Notes/Comments:

K-875001 + SEWing AD  
AG 5-30-14  
in Plasmid BOX

NEXT STEP:

ElectroporationContinued on back? Yes ☐; No ☒

Name(s): Anna Barvey  
Sienna Tackett

## Electroporation

Page: 187

DNA USED B875001  
B75001 sewing AD  
Reagents: \_\_\_\_\_

### Cell Preparation

Competent cells thawed? Yes ☒ No \_\_\_\_\_ Amount? \_\_\_\_\_

### Electroporation

Electroporation cuvette chilled? Yes ☒ No \_\_\_\_\_

Amount of <sup>LB</sup> SOC added to culture tubes: 1 ml

DNA placed in m/f tubes? Yes ☒ No \_\_\_\_\_

Placed on ice? Yes ☒ No \_\_\_\_\_

Amount of DNA mixed with competent cells: 2 µl

Notes: no arc!

Voltage used for electroporation: 400

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No \_\_\_\_\_

Amount added: 1 ml

Cells shaken at 37°C? Yes ☒ No \_\_\_\_\_

Start time: 7:50 End time: 8:50

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells spun in centrifuge (10-15s)? Yes \_\_\_\_\_ No \_\_\_\_\_

s/n decanted off? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells resuspended? Yes \_\_\_\_\_ No \_\_\_\_\_

Amount of solution: \_\_\_\_\_

Dilutions performed:

10<sup>-1</sup> Yes ☒ No \_\_\_\_\_

10<sup>-2</sup> Yes ☒ No \_\_\_\_\_

10<sup>-3</sup> Yes ☒ No \_\_\_\_\_

10<sup>-4</sup> Yes ☒ No \_\_\_\_\_

10<sup>-5</sup> Yes ☒ No \_\_\_\_\_

10<sup>-6</sup> Yes ☒ No \_\_\_\_\_

10<sup>-7</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

10<sup>-8</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

10<sup>-9</sup> Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: KB75001

Location of products: Incubator in Phillips on the left

Next steps: liquid culture

Signature: Sienna Tackett

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

A, B, + C / A<sup>0</sup>, B<sup>0</sup>, C<sup>0</sup>  
in  
Primer box  
in -20°C



#1  $\rightarrow$  A

#2  $\rightarrow$  B

#3  $\rightarrow$  C

BTS001

A<sup>0</sup>

B<sup>0</sup>

C<sup>0</sup>

A<sup>-1</sup>

$\downarrow$

A<sup>-2</sup>

A<sup>-3</sup>

A<sup>-4</sup>

A<sup>-5</sup>

A<sup>-6</sup>

NOTE: B<sup>-2</sup> sizzled

- All ~~pellets~~ pellets were grey.

Date and Time: 6/2/14

Lab Technicians(s) involved: Daniel Schreder

Protocol: cookin' up some <sup>Liquid</sup> Agarose & <sup>reusing</sup> the liquidReagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

LB broth from previous week; will autoclave

Procedure (with applicable notes):

11.5g <sup>Agar</sup> ~~Agarose~~ in 500mL H<sub>2</sub>O } autoclave  
 → reusing LB broth

• will pour agar [w/ 100mL of chloro] into plates

• will pour 5mL LB broth [w/ 3mL of PBS & cells] into conical tubes

Results:

Location of product: ~~on table~~ ~~on table~~ ~~in worm room~~ <sup>Librethel cells in worm room; agar plate on desk</sup>  
 Label on product: <sup>Name & date</sup>  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Ligation &amp; electroporation

Continued on back? Yes ☐; No ☐

Date and Time: 6/2/14 Performed by: [Signature]

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 3
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1100  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 5.5  $\mu$ L
4. Subtract #3 from #2 1094.5  $\mu$ L
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

304450  $\rightarrow$  1.)  $\frac{90.2 \text{ mg}}{1 \text{ mL}} \rightarrow \frac{.0902 \text{ mg}}{1 \text{ mL}}$  2.)  $\frac{1 \text{ mL}}{0.0902 \text{ mg}} = 11.09 \frac{\text{mL}}{\text{mg}}$

$$k. 215104 \rightarrow 1.) \frac{420 \text{ mg}}{1 \text{ mL}} \rightarrow \frac{0.42 \text{ mg}}{1 \text{ mL}} \quad 2.) \frac{1 \text{ mL}}{0.42 \text{ mg}} = 2.38 \frac{\text{mL}}{\text{mg}}$$

$$k215010 \rightarrow 1.) \frac{71.7 \text{ mg}}{1 \text{ mL}} \rightarrow \frac{40717 \text{ mg}}{1 \text{ mL}} \quad 2.) \frac{1 \text{ mL}}{0.0717 \text{ mg}} = 13.95 \frac{\text{mL}}{\text{mg}}$$

for restriction  
digest

Date and Time: 4/2/14 Performed by: \_\_\_\_\_**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB Buffer 2.1
- o DNA 304450, k215010, k215104 (from 5/4/14 prep)
- o Enzyme SPE I & EORI

| MATERIALS | TYPE          | AMOUNT USED    |
|-----------|---------------|----------------|
| Buffer    | NEB 2.1       | 2.5 ml         |
| DNA       | Joh450        | 11.1 ml        |
| Enzyme(s) | SPE I<br>EORI | 1.5 ml of each |
| Water     | DEPC          | 8.9 ml         |

Total Reaction Volume 25 ml

6.5 ml from Joh450  
the rest from Joh4502 because  
I ran out. I added the 3 ml more  
just to be safe changing  
the rest

Construct a table with this format for each reaction that you do today.

|        |         |                            |
|--------|---------|----------------------------|
| Buffer | NEB 2.1 | 2.5 ml                     |
| DNA    | k215010 | <del>11.1 ml</del> 2.38 ml |
| Enzyme | SPE I   | 1.5 ml                     |
|        | EORI    | 1.5 ml                     |
| Water  | DEPC    | 17.1 ml                    |

Total 25 ml

9 ml from k215010 #1, the rest  
from k215010 #2 because  
I ran out

|   |         |         |
|---|---------|---------|
| " | NEB 2.1 | 2.5 ml  |
| " | k215010 | 17.0 ml |
| " | SPE I   | 1.5 ml  |
| " | EORI    | 1.5 ml  |
| " | DEPC    | 5.5 ml  |

Total 25 ml

Start Time of Incubation: 10:40

End Time of Incubation: 11:40

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°

Method used for Quenching the Reaction (*different for different enzymes*)

65° for 2 min

Where did you store your finished product and what did you label it?

In -4°; green rack; 704450 R.D. 6/2/14  
K215010  
K215010

NEXT STEP:



Date and Time: 6/3/14; 8:30Performed by: Daniel Scrabble

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 3
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1100  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 5.5  $\mu$ L
4. Subtract #3 from #2 1094.5  $\mu$ L
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

$$504450 \quad \frac{50}{28.0} = 1.76$$

u215104 50/24.4 = 2.05

$$1.215010 \quad 50/13.5 = 3.7$$

Date and Time: 6/3/14  
 Protocol: Ligation  
 Reagents: T4 DNA Ligase Buffer; T4 DNA Ligase

Lab Technicians(s) involved: Dan Schwach

# Procedure (with applicable notes):

|                      | K21510   | K215104  | Control #1 | Control #2 |
|----------------------|----------|----------|------------|------------|
| DEPC water           | 11.54 ml | 13.14 ml | 17.00      |            |
| T4 DNA Ligase Buffer | 2 ml     | 2 ml     | 2 ml       |            |
| T4 DNA Ligase        | 1 ml     | 1 ml     | 1 ml       |            |
| Vector DNA           | 1.76 ml  | 1.76 ml  | 1.76 ml    |            |
| Inserted DNA         | 3.7 ml   | 2.05 ml  | —          |            |

→ mix + 10 minutes for reaction at Room temp & then electroporation

# Results:

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

# NEXT STEP:

Continued on back? Yes ☐; No ☐

Name(s): Daniel Schreier

## Electroporation

Page: 193

Reagents: 100 µg 30450, 10 µg 215104, 10 µg 215040

### Cell Preparation

Competent cells thawed? Yes X No      Amount?     

### Electroporation

Electroporation cuvette chilled? Yes X No     

Amount of LB SOC added to culture tubes: 1 mL

DNA placed in m/f tubes? Yes X No     

Placed on ice? Yes X No     

Amount of DNA mixed with competent cells: 2 µL

Voltage used for electroporation: 1001

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes X No     

Amount added: 1 mL

Cells shaken at 37°C? Yes X No     

Start time:      End time:     

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes      No     

Cells spun in centrifuge (10-15s)? Yes      No     

s/n decanted off? Yes      No     

Cells resuspended? Yes      No     

Amount of solution:     

Dilutions performed:

10<sup>-1</sup> Yes      No     

Volumes:     

10<sup>-2</sup> Yes      No     

Volumes:     

10<sup>-3</sup> Yes      No     

Volumes:     

10<sup>-4</sup> Yes      No     

Volumes:     

10<sup>-5</sup> Yes      No     

Volumes:     

10<sup>-6</sup> Yes      No     

Volumes:     

10<sup>-7</sup> Yes      No     

Volumes:     

10<sup>-8</sup> Yes      No     

Volumes:     

10<sup>-9</sup> Yes      No     

Volumes:     

Labels on products: extremal

Location of products:     

Next steps:     

Signature:     

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

Not successful 6/5/14

I already streaked them onto a plate; in warm room labeled update 6/3/14 D.S. & then DNA strand

# Gel Electrophoresis

Page p.194

Name: Jacob Soda

Date: 6/1/14

Time: 4:00 pm

% Agarose: 1%

Lane Reagents

|   |  |                     |   |
|---|--|---------------------|---|
| 1 |  |                     |   |
| 2 |  |                     |   |
| 3 |  |                     |   |
| 4 | 2.5 mL K314200 (p.126)                       | + .5 mL loading dye |   |
| 5 | 2.5 mL K875001 (p.130)                       | + .5 mL             | " |
| 6 | 2.5 mL B (p.181)                             | + .5 mL             | " |
| 7 | 2.5 mL A (p.181)                             | + .5 mL             | " |
| 8 | Ladder already has loading dye in it<br>1 mL |                     |   |

.1-10 kb

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Gel started at 4:53 pm at 100V end at 5:52 pm

starting at 4:20 pm bands didn't seem separated enough  
start at 6:02 pm run to 615 to separate the bands more

Reagents used:

.50 g agarose LE  
50 mL 1x TAE buffer  
2.5 mL gel red

A = sewing PCR product w/ flanking primers 562 bp  
B = sewing PCR product w/o flanking primers

K875001 = PCR product (labeled AB on pg.130) 85 bp  
K314200 = PCR product (labeled D on pg.126) 477 bp

Note: K875001 and K314200 from pages 124 and 126 are used up, so I used the PCR products from earlier (p.126, p.130)

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

### Results

The band in between 400 and 500bp looks the same for sewing PCR product A and K34200, so I do not ~~think the~~ <sup>think the</sup> sewing PCR worked, since the product should have a higher bp length. There did not appear to be a 85bp band in the sewing PCR product A, so K34200 is not in the product = good.

Failed

NEXT STEP: Consult Matt/Anna to see if sewing PCR has to be redone

Name(s): Zachary Binner  
Jacob JodanDate and Time: 6/6/14Liquid CultureAmount of LB used: 5mls per tube, 30ml totalPart Used: K215104, 504450Antibiotic Used: Amp, KanAmount of Antibiotic Used: 10ml, 3.5mlConcentration of Antibiotic: 100mg/ml, 35mg/mlLabel on Product: Part, antibiotic, Date, InitialsLocation of Product: warm room, Eppendorf shakerNext Step: mini prep

## Zymo Mini Prep

Name(s): Daniel Schuel  
 Date and Time: 6/7/11 ; 8:10 AM

Check off as you  
complete the steps

**Procedure:**

Bacteria used: k215104 [AMP], J04450 [Kan]

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☐
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☐
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☐
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zippy Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 30 $\mu$ L Zippy Elution Buffer to column..... ☐
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: 6/7/11 J04450 or k215104 miniprep  
 Location of product: -20001 freezer plastic box

Deviations from Procedure and other Notes: \*used Qigen Neutralization Buffer [v3]

NEXT STEP: Qbit & then R.D.

Continued on back? Yes ☐; No ☐



Gel Electrophoresis

Name: David Schmidt, Josh  
Date: 5/10/14  
Time: 8:15 AM

% Agarose: 1

Lane Reagents

|   |        |  |
|---|--------|--|
| 1 |        |  |
| 2 | Jc     |  |
| 3 | Jb     |  |
| 4 | Ja     |  |
| 5 | Kc     |  |
| 6 | Kb     |  |
| 7 | Ka     |  |
| 8 | Ladder |  |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

2.5 Ladder  
3.5 sample DNA

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

## Results

Good; looks like miniprep was all good

NEXT STEP: Restriction digest to get very  
large quantities of h215104

Date and Time: \_\_\_\_\_ Performed by: Daniel Schuch / Josh Leavine

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 6
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1700  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 8.5
4. Subtract #3 from #2 169.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

| Sample Name      | Page | Concentration         |
|------------------|------|-----------------------|
| K2S104 [A] mwpmp |      | 258 $\mu\text{L/mL}$  |
| " [B] "          |      | 229 $\mu\text{L/mL}$  |
| " [C] "          |      | 245 $\mu\text{L/mL}$  |
| Jawuso [A] "     |      | 87.2 $\mu\text{L/mL}$ |
| " [B] "          |      | 121 $\mu\text{L/mL}$  |
| " [C] "          |      | 92.7 $\mu\text{L/mL}$ |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |
|                  |      |                       |

~~At least 2000~~

Date and Time: 6/10/14Performed by: Amel Schroder, Josh Levene**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB Buffer 2.1
- o DNA 51456 (ABC), K25104 (ABC)
- o Enzyme SPE I, EcorI

| MATERIALS | TYPE           | AMOUNT USED                |
|-----------|----------------|----------------------------|
| Buffer    | NEB 2.1        | 2.5 $\mu$ l                |
| DNA       | 51456          | 15 $\mu$ l                 |
| Enzyme(s) | SPE I<br>EcorI | 1.5 $\mu$ l<br>1.5 $\mu$ l |
| Water     | DEPC           | 4.5 $\mu$ l                |

Total Reaction Volume 25 ml

Note: 7/15/14 probably should have done 10  $\mu$ l 51456 & 9.5  $\mu$ l DEPC (same formula for all samples)

Construct a table with this format for each reaction that you do today.

| Materials | Type           | Amnt. used                 |
|-----------|----------------|----------------------------|
| Buffer    | NEB 2.1        | 2.5 $\mu$ l                |
| DNA       | K25104         | 15 $\mu$ l                 |
| Enzyme(s) | SPE I<br>EcorI | 1.5 $\mu$ l<br>1.5 $\mu$ l |
| Water     | DEPC           | 4.5 $\mu$ l                |

control group #1

Buffer NEB 2.1 2.5 ml  
DNA 51456 ~~2~~ 2 ml  
Enzyme spe I 1.5 ml  
water DEPC 19 ml

control group #2

Buffer NEB 2.1 2.5 ml  
DNA 51456 2 ml  
Enzyme EcorI 1.5 ml  
water DEPC 19 ml

control group #4

" " 2.5 ml  
" K25104 2 ml  
" EcorI 1.5 ml  
" DEPC 19 ml

control group #3

" " 2.5 ml  
" K25104 2 ml  
" spe I 1.5 ml  
" DEPC 19 ml

Start Time of Incubation: 10:55 AM

End Time of Incubation: 11:55 AM

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

65°C for 20 minutes

Where did you store your finished product and what did you label it?

in freezer; green rack labeled with date  
25, K, C#1239

NEXT STEP: run gel & gel extract

Name(s): Zach BirnerDate and Time: 6/10/14Liquid CultureAmount of LB used: 5ml each x 8 tubesPart Used: DH5 $\alpha$ , Nisile 1917Antibiotic Used: noneAmount of Antibiotic Used: NAConcentration of Antibiotic: NALabel on Product: Part, 6/10/14, 2B (orange tape)Location of Product: warm room or shaker

Next Step: \_\_\_\_\_

Date and Time: 6/10/14 Performed by: Jacob Jodet  
5:30 pm

### Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

### PROCEDURE

#### Material Location

- Buffer NEB 2.1 from the biobrick box
- DNA K875001 from page 152 in the plasmid box
- Enzyme Pst I, Xba I from the biobrick box  
 used the amounts from p.173

| MATERIALS | TYPE    | AMOUNT USED |
|-----------|---------|-------------|
| Buffer    | NEB 2.1 | 2.5 mL      |
| DNA       | K875001 | 7.35 mL     |
| Enzyme(s) | Xba I   | 1 mL        |
|           | Pst I   | 1 mL        |
| Water     | DEPC    | 13.15 mL    |

Total Reaction Volume 25 mL

Construct a table with this format for each reaction that you do today.



Start Time of Incubation: 5:39

End Time of Incubation: 6:39

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

heat inactivation 65°C for 20 min.

Where did you store your finished product and what did you label it?

201 (on top) 15875001 digested 6/10/14

In plasma box

~~labeled~~

I transferred  
50 mL Xba I and 50 mL pTS1 per Dr. Henkel  
to microcentrifuge tubes and put them in the biobrick assembly  
box, use these enzymes so we don't have to take them from the  
stock everytime

NEXT STEP:

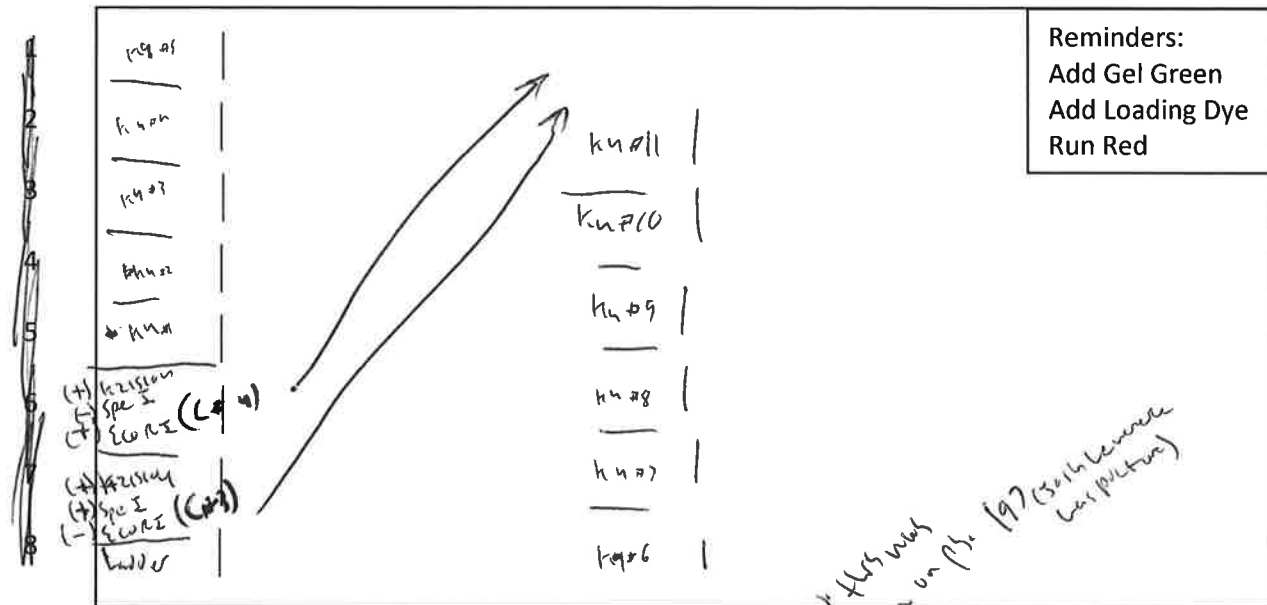
# Gel Electrophoresis

Page 202

Name: David S. ...  
 Date: 6/11/14  
 Time: 10:00 AM

% Agarose: 1

Lane Reagents



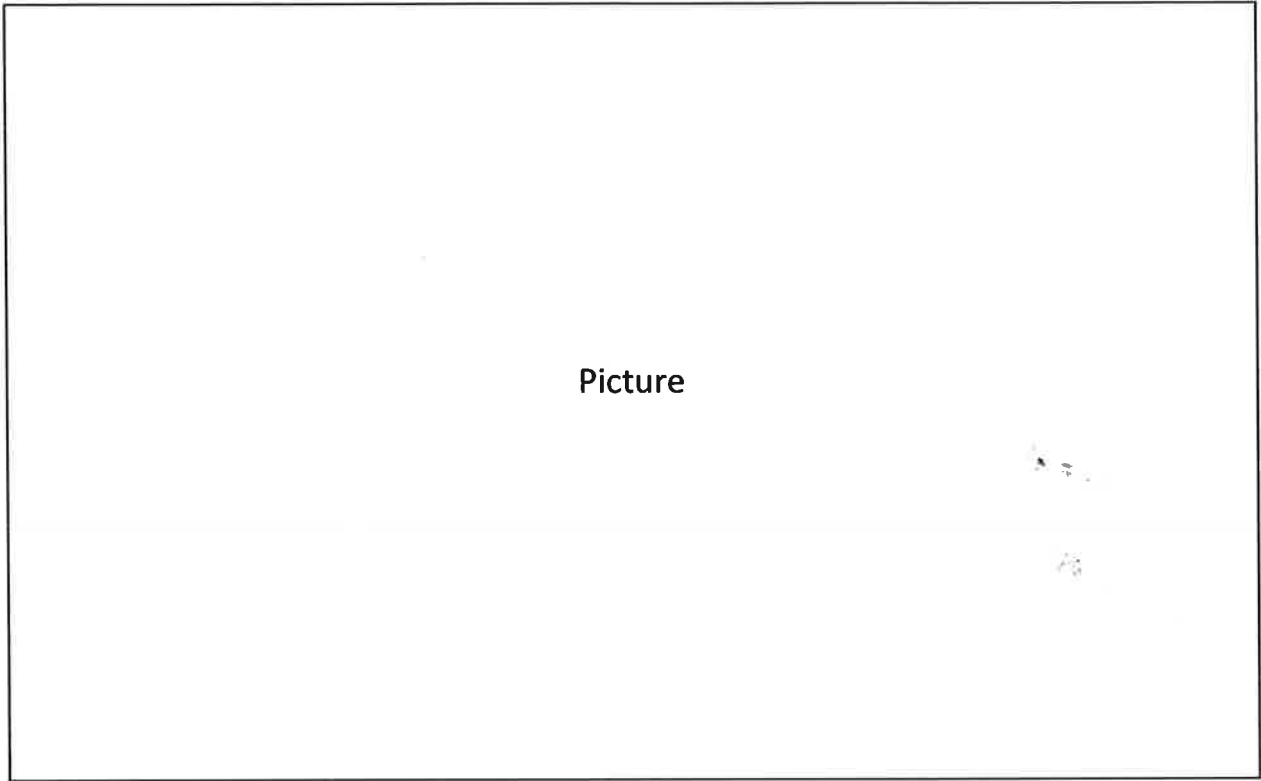
Reagents used:

\* 1 kb → 10 µl of DNA for each sample + 2 loading dye

1.5 g agarose  
 100 mL 1x TAE Buffer  
 7.5 µl Gel Red

Gel Electrophoresis

Names: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

NEXT STEP: Gel extract

Date and Time: 6/11/14 12:45 PM Performed by: Zach Binner, Daniel Schroeder  
Josh Leverance

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |                   |   |                    |                    |                    |                    |                    |                    |                    |                    |
|-------------------|-------------------|---|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Empty Tube        | Sample            |   | 1.061              | 1.063              | 1.045              | 1.048              | 1.055              | 1.068              | 1.070              | 1.061              |
| Sample            | Tube + Gel weight | 9 | K <sub>4</sub> (b) | K <sub>4</sub> (c) | K <sub>4</sub> (d) | K <sub>4</sub> (e) | K <sub>4</sub> (f) | K <sub>4</sub> (g) | K <sub>4</sub> (h) | K <sub>4</sub> (i) |
| Tube + Gel weight | Empty tube weight |   | 1.18               | 1.26               | 1.21               | 1.20               | 1.23               | 1.32               | 1.20               | 1.19               |
|                   | Gel Weight        |   | 119 $\mu$ g        | 197 $\mu$ g        | 165 $\mu$ g        | 152 $\mu$ g        | 175 $\mu$ g        | 252 $\mu$ g        | 130 $\mu$ g        | 129 $\mu$ g        |
|                   | Buffer QG         |   | 357 $\mu$ L        | 591 $\mu$ L        | 495 $\mu$ L        | 456 $\mu$ L        | 525 $\mu$ L        | 756 $\mu$ L        | 390 $\mu$ L        | 387 $\mu$ L        |
|                   | Isopropanol       |   | 119                | 197                | 165                | 152                | 175                | 252                | 130                | 129                |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product:

Location of Product:

Next Step:

Date and Time: 6/11/14 12:00 PM Performed by: Zach Birner, Daniel Schroeder, Josh Lerencee

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |                      |                    |                    |                    |                    |                    |  |  |  |
|-------------------|----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--|--|--|
| Sample            | 1.059                | 1.055              | 1.065              | 1.060              | 1.070              | 1.08               |  |  |  |
| Tube + Gel weight | K <sub>4</sub> (J)   | K <sub>4</sub> (K) | K <sub>4</sub> (L) | K <sub>4</sub> (M) | K <sub>4</sub> (N) | K <sub>4</sub> (O) |  |  |  |
| Empty tube weight | <del>1.27</del> 1.27 | 1.21               | 1.22               | 1.23               | 1.36               | 1.22               |  |  |  |
| Gel Weight        | 211 mg               | 155 mg             | 155 mg             | 170 mg             | 290 mg             | 140 mg             |  |  |  |
| Buffer QG         | 633 $\mu$ L          | 465 $\mu$ L        | 465 $\mu$ L        | 510 $\mu$ L        | 870 $\mu$ L        | 420 $\mu$ L        |  |  |  |
| Isopropanol       | 211 $\mu$ L          | 155 $\mu$ L        | 155 $\mu$ L        | 170 $\mu$ L        | 290 $\mu$ L        | 140 $\mu$ L        |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product:

Location of Product:

Next Step:

Date and Time: 6/11/14 12:00 PM Performed by: Zach Bruner, Daniel Schroeder, Josh Leverette

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

*Empty Tube Weight*  
*Sample Weight*  
*Tube + Gel Weight*

|                   |   |                                  |                                  |                                  |                                  |                                  |                                  |                                  |   |
|-------------------|---|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|---|
| Sample            | <del>1.07g</del>                        | 1.07g                            | 1.07g                            | 1.05g                            | 1.06g                            | 1.07g                            | 1.06g                            | 1.07g                            |   |
| Tube + Gel weight | <del>K<sub>4</sub>(a<sub>2</sub>)</del> | K <sub>4</sub> (b <sub>2</sub> ) | K <sub>4</sub> (c <sub>2</sub> ) | K <sub>4</sub> (d <sub>2</sub> ) | K <sub>4</sub> (e <sub>2</sub> ) | K <sub>4</sub> (f <sub>2</sub> ) | K <sub>4</sub> (g <sub>2</sub> ) | K <sub>4</sub> (h <sub>2</sub> ) | I |
| Empty tube weight |   | 1.20                             | 1.24                             | 1.18                             | 1.22                             | 1.29                             | 1.26                             | 1.20                             |   |
| Gel Weight        |   | 130mg                            | 190mg                            | 130mg                            | 160mg                            | 220mg                            | 200mg                            | 130mg                            |   |
| Buffer QG         |   | 390μL                            | 570μL                            | 390μL                            | 480μL                            | 660μL                            | 600μL                            | 390μL                            |   |
| Isopropanol       |   | 130μL                            | 190μL                            | 130μL                            | 160μL                            | 220μL                            | 200μL                            | 130μL                            |   |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100μL per 100mg; so 3 volumes is 300μL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800μL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750μL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30μL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product:

Location of Product:

Next Step:

Date and Time: 6/11/14 12:00 PM Performed by: Zach Birn, Daniel Schroeder, John Lawrence

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |   |   |   |             |          |             |  |  |  |
|-------------------|---|---|---|-------------|----------|-------------|--|--|--|
| Sample            |   |   |   | 1.05g       |          | 1.07g       |  |  |  |
| Tube + Gel weight | J | k | L | $K_4(M_2)$  | $K_4(N)$ | $K_4(O_2)$  |  |  |  |
| Empty tube weight |   |   |   | 1.19        |          | 1.23        |  |  |  |
| Gel Weight        |   |   |   | 140mg       |          | 160mg       |  |  |  |
| Buffer QG         |   |   |   | 420 $\mu$ L |          | 480 $\mu$ L |  |  |  |
| Isopropanol       |   |   |   | 140 $\mu$ L |          | 160 $\mu$ L |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.

6. Add 1 volume of isopropanol to each tube and mix gently.

7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)

10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product:

Location of Product:

Next Step:

Date and Time: 4:10 pm 6/11/17  
Protocol: Ligation  
Reagents:

Lab Technicians(s) involved: Jacob Sadat

Procedure (with applicable notes):

Load <sup>74</sup> DNA Ligase  
K875001 Digest: 1.35 mL  
Sewing PCR + AD Digest: 1.62 mL  
DePC: 14.03 mL  
2 mL of 10XT4 DNA Ligase Buffer

Results:

Location of product: Plasmid box  
Label on product: Ligation of sewing PCR product AD and K875001  
End Notes/Comments:

NEXT STEP:

Electroporation

Continued on back? Yes ☐; No ☐



6/11/14

Name(s): Seob Jodat**Electroporation**Page: 208Auna GarveyReagents: <sup>(ligated)</sup> DNA: K875001 and SEWing PCR product AD**Cell Preparation**Competent cells thawed? Yes X No   Amount? 4 X 10<sup>10</sup> 1917**Electroporation**Electroporation cuvette chilled? Yes X No   Amount of <sup>LB</sup> ~~SOC~~ added to culture tubes: 1 mLDNA placed in m/f tubes? Yes X No   Placed on ice? Yes X No   Amount of DNA mixed with competent cells: 2  $\mu$ LVoltage used for electroporation: EC0 1\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.SOC immediately added after electroporation? Yes X No   Amount added: 1 mLCells shaken at 37°C? Yes X No   Start time: 5:15pm End time:   **Serial Dilution**Cells transferred to 1 mL m/f tube? Yes    No   Cells spun in centrifuge (10-15s)? Yes    No   s/n decanted off? Yes    No   Cells resuspended? Yes    No   Amount of solution:   **Dilutions performed:**10<sup>-1</sup> Yes 1,3 No 2,410<sup>-2</sup> Yes 2,4 No 1,310<sup>-3</sup> Yes 1,3 No 2,410<sup>-4</sup> Yes 2,4 No 1,310<sup>-5</sup> Yes    No   10<sup>-6</sup> Yes    No   10<sup>-7</sup> Yes    No   10<sup>-8</sup> Yes    No   10<sup>-9</sup> Yes    No   Volumes:   Volumes:   Volumes:   Volumes:   Volumes:   Volumes:   Volumes:   Volumes:   Volumes:   Labels on products: K875001 + SEW PCR AD Ec01/Nigle 1917 6-11-14Location of products: Warm RoomNext steps: Pick coloniesSignature:   From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

Name(s): Zach BirnerDate and Time: 6/12/14 4:55Liquid CultureAmount of LB used: 5ml x 4 culturesPart Used: 875001 + Sew PCR in ecoli Nissle 1917Antibiotic Used: chloroAmount of Antibiotic Used: 1ml per tubeConcentration of Antibiotic: 10 ~~mg~~ ng/ml in culture  
(50mg/ml on bottle)Label on Product: 875001 + Sew PCR, Nissle 1917, #10<sup>8</sup>, 6/12/14, chloro, ZBLocation of Product: warm room shaker, put in at 6:50 PMNext Step: glycerol stock

Name(s): Zach BirnerGlycerol stockDate and Time: 6/13/14 12:00PMLiquid Culture

Amount of <sup>culture</sup> LB used: 500ml each stock x 6 stocks + 500ml 50% glycerol x 6  
 Part Used: 87001 new PCR in Nissle 1917 ( $\approx 312.5\text{ml } 80\% \text{ glycerol} + 187.5\text{ml dH}_2\text{O}$ )  
 Antibiotic Used: chloro (in the culture)  
 Amount of Antibiotic Used: \_\_\_\_\_  
 Concentration of Antibiotic: \_\_\_\_\_

diluting  
80% glyceroltotal glycerol  
needed = 3ml  
in 3ml H<sub>2</sub>O $\frac{3}{18} = 3.75\text{ml}$   
80% glycerol  
+ 2.25ml  
H<sub>2</sub>O

Label on Product: -80°C 87001, in 600ml Nissle 1917, # (dilution) 6/13/14, 2B  
 Location of Product: -80°C, Plasmid stock box: GEM 2013  
 Next Step: \_\_\_\_\_

Name(s): Ryan GeorgeDate and Time: 6/16/14 8:40pm

Start: 11:40

Finish: 12:15

Liquid Culture

- 1 Amount of LB used: 5 ml x 4 cultures
- 2 Part Used: B0034
- 3 Antibiotic Used: Amp
- 4 Amount of Antibiotic Used: 10 ml/tube
- 5 Concentration of Antibiotic: 100 µg/ml in culture

- 1 5 ml x 4 cultures
- 2 K215104
- 3 Amp
- 4 10 ml/tube
- 5 100 µg/mL in culture

- 1 5 ml x 4 cultures
- 2 K215002
- 3 Amp
- 4 10 µL/tube
- 5 100 µg/mL in culture

OverLabel on Product: Part #, RG 6/16 (All in red sharpie on side)  
BeckmanLocation of Product: Warm room shaker

Next Step: \_\_\_\_\_

- 1 5 ml  $\times$  4 cultures
- 2 K 206000
- 3 chloro
- 4 1  $\mu$ l / tube
- 5 10  $\mu$ g/ml in culture

## Zymo Mini Prep

Name(s): ANNA GARVEY, Matt Mortensen  
 Date and Time: 10-17-14

Check off as you  
complete the steps

**Procedure:** Chloro Amp Amp Amp  
 Bacteria used: K206000, K215002, B0034, K215004

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☒  
 Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zippy Wash Buffer to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 30 $\mu$ L Zippy Elution Buffer to column..... ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: Spact #1 (Resistance) Plasmid p.212  
 Location of product: Plasmid Box

Deviations from Procedure and other Notes:



NEXT STEP:

Continued on back? Yes ☐; No ☒

Date and Time: 6-17-14 2:00pm Performed by: MATTHEW MORTENSEN & ANNA GARVEY

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed 10
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]



Date and Time: 10-17-14 2:30pm Performed by: ANNA GARVEY + MATTHEW MORTENSEN

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer NEB 2.1
- o DNA K215004, 80034, K215009, K215002
- o Enzyme pst I, spe I, xba I

"4"

| MATERIALS | TYPE                    | AMOUNT USED |
|-----------|-------------------------|-------------|
| Buffer    | NE Buffer 2.1           | 5uL         |
| DNA       | p212<br>2ug K215104 B   | 4.20 uL     |
| Enzyme(s) | XbaI<br><del>PstI</del> | 2uL<br>2uL  |
| Water     | DEPC                    | 36.8 uL     |

x3

Total Reaction Volume 50 uL

Construct a table with this format for each reaction that you do today.

~~14.28 mg~~

"3"

|                  |          |
|------------------|----------|
| B0024 B p212 2ug | 19.80 uL |
| NE Buffer 2.1    | 5 uL     |
| Enzyme SpeI      | 2 uL     |
| PstI             | 2 uL     |
| Dep C            | 21.2 uL  |

x1

"0"

|                      |          |
|----------------------|----------|
| NE Buffer 2.1        | 5 uL     |
| K206000 B (p212) 2ug | 11.90 uL |
| Enzyme SpeI          | 2 uL     |
| PstI                 | 2 uL     |
| Dep C                | 29.1 uL  |

x1

"2"

|                      |          |
|----------------------|----------|
| NE Buffer 2.1        | 5 uL     |
| K215002 A (p212) 2ug | 11.05 uL |
| Enzyme SpeI          | 2 uL     |
| PstI                 | 2 uL     |
| Dep C                | 29.95 uL |

x1

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 65°

Method used for Quenching the Reaction (*different for different enzymes*)

Ø

Where did you store your finished product and what did you label it?

iGEM Primer Box | <Part> Digest p214 AG 6-17-14

NEXT STEP:

Name(s): Jacob JodatDate and Time: 5:30pm 6/17/14Liquid CultureAmount of LB used: 5mlPart Used: K875001 and K314200 in vial #17 used all 4 samples from glycerol stockAntibiotic Used: chloramphenicolAmount of Antibiotic Used: 1 mLConcentration of Antibiotic: 10mg/mL

labels

4a = Stock #4 dilution 10

~~3a~~ stock #4 "

3a

3b

2a

2b

2c

labeled in glycerol stock

Label on Product: ~~SEWPER~~ <sup>plasmid</sup> product from vial #17, #55, 6/17/14 JGMLocation of Product: Warm RoomNext Step: miniprep

## Zymo Mini Prep

Name(s): Jacob JodatDate and Time: 2:30 pm 6/18/14Check off as you  
complete the steps**Procedure:**Bacteria used: Transformed visse 1917 (contains K12501 and K314200) I used 2a, 2b, 2c, 3a, 3b, 4a from the glycerol stock1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐

Note: This step differs from the printed instructions

2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☐Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L3. Add 100 $\mu$ L 7X Lysis Buffer..... ☐

Invert 4-6 times (less than 5 minutes until next step)

4. Add 350 $\mu$ L Neutralization Buffer..... ☐

Inverting 4-6 times immediately

5. Centrifuge 6min at 13000rpm..... ☐6. Pipet supernatant into a QIAprep spin column..... ☐

Centrifuge 30-60sec then discard flow through

Note: If multiple cultures of the same type are used, they are combined in this step

7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☐

Centrifuge 30-60sec and then discard flow through

8. Add 400 $\mu$ L of Zippy Wash Buffer to column..... ☐

Centrifuge 30-60sec and then discard flow through

9. Transfer column to new microcentrifuge tube..... ☐10. Add 30 $\mu$ L Zippy Elution Buffer to column..... ☐

Let stand for 60sec then centrifuge for 60sec

11. Discard column and save centrifuge tube with flow through..... ☐12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐Label on centrifuge tube: on top 216 and either 2a, 2b, 2c, 3a, 3b, or 4aLocation of product: plasmid box

Deviations from Procedure and other Notes:

NEXT STEP: Gel electrophoresisContinued on back? Yes ☐; No ☐

## Gel Electrophoresis

Name: Jacob J. J.Date: 6/18/18Time: 9:25 pm% Agarose: 1%

Lane Reagents

|   |                               |
|---|-------------------------------|
| 1 |                               |
| 2 | 2.5 mL 2C 1.5 loading dye     |
| 3 | 2.5 mL 2b "                   |
| 4 | 2.5 mL 2a "                   |
| 5 | 2.5 mL 3b "                   |
| 6 | 2.5 mL 3a "                   |
| 7 | 2.5 mL 4a "                   |
| 8 | Ladder 1 mL 2 log (0.1-10 kb) |

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Start 10:01 pm Stop 10:56 pm

Reagents used:

150g agarose

50 mL 1x TAE buffer

2.5 mL gel red

Gel made, electrophoresis still has to be done 4:42 pm  
Gel in 4°C fridgeNote: the miniprep product has some loading dye in it, I put it in the wrong tubes initially, that's why they are blue  
(from page 216)

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

## Results

bands looked between 200-800 bp, not sure if it is the right thing...

NEXT STEP:

# Gel Electrophoresis

Page 218

Name: Daniel Schaefer

Date: 6/23/19

Time: 8:30 AM

% Agarose: 1

Lane Reagents

|   |                  |   |
|---|------------------|---|
| 1 | Jones 001 (R.D.) | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | Jones 004        |   |
| 3 | Jones 003        |   |
| 4 | Jones 002        |   |
| 5 | Jones 001        |   |
| 6 | Control #2       |   |
| 7 | Control #1       |   |
| 8 | Ladder           |   |

Reagents used:

Jones 001 of DNA for each sample + 2 µl loading dye

Handwritten notes:

Control #2 = Jones 001

Control #1 = Jones 002

Control #3 = Jones 003

Control #4 = Jones 004

Control #5 = Jones 005

Control #6 = Jones 006

Control #7 = Jones 007

Control #8 = Jones 008

Control #9 = Jones 009

Control #10 = Jones 010

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

phone died so was  
not able to take a picture  
but everything looked good

NEXT STEP:

- \* storing in sliding refrigerator
- \* gel extraction



Date and Time: \_\_\_\_\_ Performed by: \_\_\_\_\_

**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | Joh450 #1              | Joh450 #2              | Joh450 #3               | Joh450 #4              | Joh450 #5              |  |  |  |  |
|-------------------|------------------------|------------------------|-------------------------|------------------------|------------------------|--|--|--|--|
| Tube + Gel weight | 1.015g                 | 0.989g                 | 1.054g                  | 1.001g                 | 0.954g                 |  |  |  |  |
| Empty tube weight | 0.918g                 | 0.906g                 | 0.936g                  | 0.926g                 | 0.914g                 |  |  |  |  |
| Gel Weight        | <del>0.097g</del> 97µg | <del>0.083g</del> 83µg | <del>0.118g</del> 118µg | <del>0.075g</del> 75µg | <del>0.040g</del> 40µg |  |  |  |  |
| Buffer QG         | 291µl                  | 249µl                  | 354µl                   | 225µl                  | 120µl                  |  |  |  |  |
| Isopropanol       | 97µl                   | 83µl                   | 118µl                   | 75µl                   | 40µl                   |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100µL per 100mg; so 3 volumes is 300µL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800µL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750µL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30µL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: Joh450 ; gel extract, 4/23/17

Location of Product: freezer ; Tempump box

Next Step: ligation &amp; electroporation

# Gel Electrophoresis

Page 220

Name: Jacob Sodal

Date: 6/29/19

Time: 3:40 pm

% Agarose: 1%

Lane Reagents

|   |  |        |                     |
|---|--|--------|---------------------|
| 1 |  |        |                     |
| 2 | 4a   | 2.5 mL | + .5 mL loading dye |
| 3 | 3b   | "      | "                   |
| 4 | 3a   | "      | "                   |
| 5 | 2c   | "      | "                   |
| 6 | 2b   | "      | "                   |
| 7 | 2a   | "      | "                   |
| 8 | ladder (10g) + .5 mL loading dye because ladder did not show up well last time<br>1 mL |        |                     |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Start Gel 4:12 pm      End at 5:10  
at 120V

Reagents used:

.51g agarose  
50 mL 1x TAE buffer  
2.5 mL gel red

redoing gel from page 217 to try to get the ladder to show up better

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

## Gel Electrophoresis

Name: Paul SchulerDate: 6/25/14Time: 8:30 AM% Agarose: 1

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

Tungso

Tungso

Tungso

Tungso

Tungso

C02

C01

Ladder

Restriction  
Digest

Reminders:

Add Gel Green

Add Loading Dye

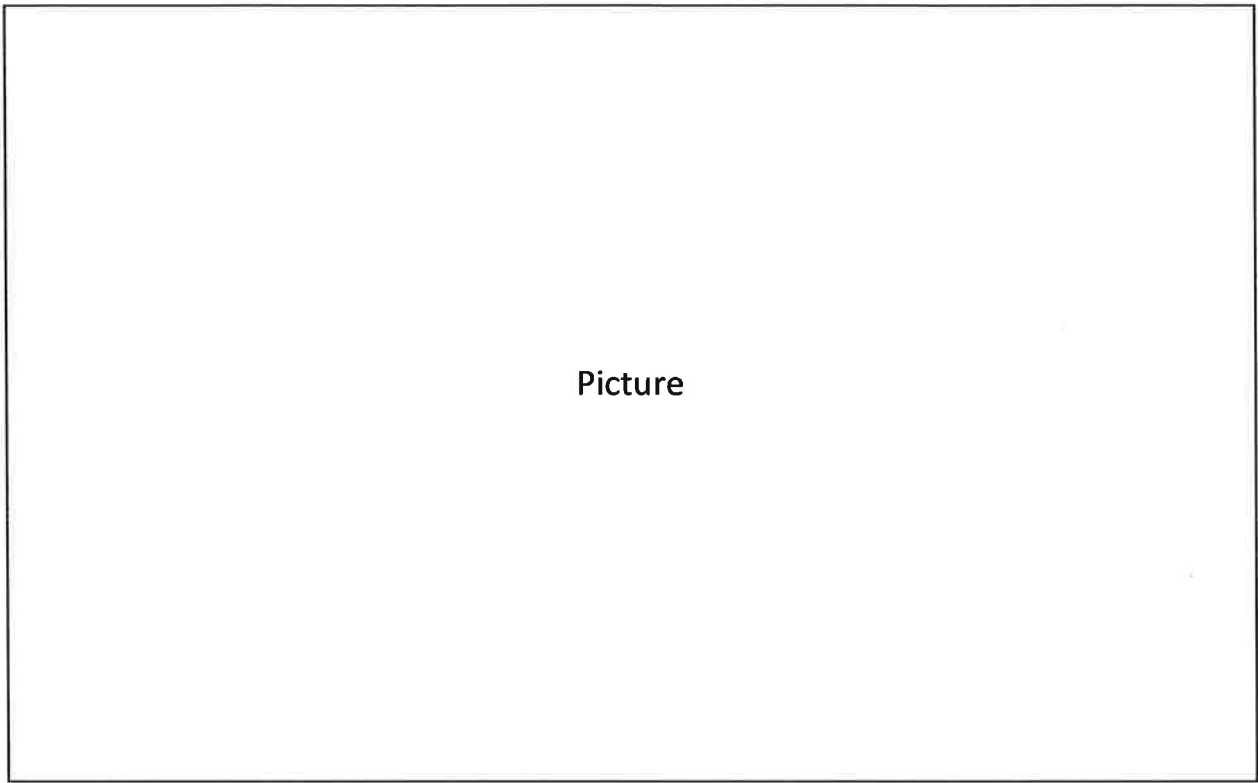
Run Red

Reagents used:

10  $\mu$ l DNA + 2  $\mu$ l loading dye2  $\mu$ l ladder

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

|            |
|------------|
| NEXT STEP: |
|------------|

Date and Time: 9:15, 6/25/14

Performed by: Daniel Schmitt

**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | Jones  | Jones  | Jones | Jones | Jones | 2/0 |  |  |  |
|-------------------|--------|--------|-------|-------|-------|-----|--|--|--|
| Tube + Gel weight | 1.055g | 1.041g | 1.02g | 1.08g | 1.04g |     |  |  |  |
| Empty tube weight | 0.914g | 0.920g | 0.91g | 0.91g | 0.94g |     |  |  |  |
| Gel Weight        | 141µg  | 121µg  | 110µg | 170µg | 100µg |     |  |  |  |
| Buffer QG         | 423µL  | 363µL  | 330µL | 510µL | 300µL |     |  |  |  |
| Isopropanol       | 141µL  | 121µL  | 110µL | 170µL | 100µL |     |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100µL per 100mg; so 3 volumes is 300µL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800µL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750µL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30µL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: Gel extracted; 6/25/14; Jones

Location of Product: Team pumpbox in freezer

Next Step: Ligation electroporation

Name(s): Jacob Solaf

Date and Time: 6:30 6/26/14

Liquid Culture

Amount of LB used: 5ml

Part Used: DH5 $\alpha$  6 cultures

Antibiotic Used: —

Amount of Antibiotic Used: —

Concentration of Antibiotic: —

Label on Product: DH5 $\alpha$ 

Location of Product: in warm room

Next Step: —

# Gel Electrophoresis

Page 224

Name: Anna Gawey

Date: 6-27-14

Time: 12:30

% Agarose: 1%

Lane Reagents

1

2

3

4

5

6

7

8

K215104 Digested (40ul/lane)

K215104 Undigested

Ladder

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:



## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

### Results

All digested material showed proper band length. The larger pieces were excized <sup>and a</sup> ~~via~~ gel extraction (p. 225) was performed

### NEXT STEP:

Gel Extraction

Date and Time: 6-27-14

Performed by: ANNA GARVEY &amp; MATT MORTENSEN

**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | 1                        | 2                    | 3     | 4     | 5     |  |  |  |  |
|-------------------|--------------------------|----------------------|-------|-------|-------|--|--|--|--|
| Tube + Gel weight | 1.18<br><del>1.057</del> | 1.18                 | 1.20  | 1.21  | 1.17  |  |  |  |  |
| Empty tube weight | 1.057                    | 1.049                | 1.053 | 1.072 | 1.055 |  |  |  |  |
| Gel Weight        | .13                      | <del>1.31</del> .131 | .147  | .138  | .115  |  |  |  |  |
| Buffer QG         | .39                      | .393                 | .441  | .414  | .345  |  |  |  |  |
| Isopropanol       | .13                      | .131                 | .147  | .138  | .115  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: K215104 Gel Extraction MWM 6-27-14

Location of Product: Primer Box

Next Step: Ligation

Date and Time: 6-27-14 2pmPerformed by: Matthew Mortensen  
Anna Garvey

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 2
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 900
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 4.5
4. Subtract #3 from #2 895.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 6-27-14Lab Technicians(s) involved: Matthew MontensenProtocol: LigationAnna Garvey

Reagents: \_\_\_\_\_

$L_1$   $L_2$   $L_3$   $N_1$   $N_2$   $N_3$   
 Procedure (with applicable notes):

|  |                      |                        |                      |                      |                    |  |
|--|----------------------|------------------------|----------------------|----------------------|--------------------|--|
| Ligase Buffer                                | 2 $\mu$ L            | 2 $\mu$ L              | 2 $\mu$ L            | 2 $\mu$ L            | 2 $\mu$ L          |  |
| T4 DNA Ligase                                | 1 $\mu$ L            | 1 $\mu$ L              | —                    | 1 $\mu$ L            | 1 $\mu$ L          |  |
| Vector<br>Insert DNA<br>30074 Digest<br>p214 | .90 $\mu$ L<br>50ng  | .90 $\mu$ L<br>50ng    | .90 $\mu$ L<br>50ng  | —                    | .9 $\mu$ L<br>50ng |  |
| Insert DNA<br>16215104 Gel<br>Extract p225   | 5.94 $\mu$ L<br>50ng | 11.88 $\mu$ L<br>100ng | 6.94 $\mu$ L<br>50ng | 5.94 $\mu$ L<br>50ng | —                  |  |
| Dep C Water                                  | 10.16 $\mu$ L        | 4.22 $\mu$ L           | 11.16 $\mu$ L        | 11.06 $\mu$ L        | 16.1 $\mu$ L       |  |

Results:

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Electroporation

Continued on back? Yes ☐; No ☐

Name(s): Matt Mortensen  
Anna Garvey

## Electroporation

Page: 228

Reagents: Ligation Products p 227

### Cell Preparation

Competent cells thawed? Yes ☒ No ☐

Amount? 6 cultures

### Electroporation

Electroporation cuvette chilled? Yes ☒ No ☐

Amount of SOC added to culture tubes: ~~400~~ 400  $\mu$ L

DNA placed in m/f tubes? Yes ☒ No ☐

Placed on ice? Yes ☒ No ☐

Amount of DNA mixed with competent cells: 3  $\mu$ L

Voltage used for electroporation: ECR1

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No ☐

Amount added: 1 mL

Cells shaken at 37°C? Yes ☒ No ☐

Start time: 4:45 End time: 5:45

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes ☒ No ☐

Cells spun in centrifuge (10-15s)? Yes ☒ No ☐

s/n decanted off? Yes ☒ No ☐

Cells resuspended? Yes ☒ No ☐

Amount of solution: 100  $\mu$ L

Dilutions performed:

*Negatives not diluted*

$10^{-1}$  Yes ☒ No ☐  
 $10^{-2}$  Yes ☒ No ☐  
 $10^{-3}$  Yes ☒ No ☐  
 $10^{-4}$  Yes ☒ No ☐  
only {  $10^{-5}$  Yes ☒ No ☐  
 $L_2$  {  $10^{-6}$  Yes ☒ No ☐  
 $10^{-7}$  Yes ☐ No ☐  
 $10^{-8}$  Yes ☐ No ☐  
 $10^{-9}$  Yes ☐ No ☐

Volumes: 200  $\mu$ L  
Volumes: 200  $\mu$ L  
Volumes: 200  $\mu$ L  
Volumes: 200  $\mu$ L  
Volumes: 200  $\mu$ L  
Volumes: 200  $\mu$ L  
Volumes: \_\_\_\_\_  
Volumes: \_\_\_\_\_  
Volumes: \_\_\_\_\_

Labels on products:  $L_2$  on  $N_2$  6-27-14 MWM (Dilution Factor?)

Location of products: Wanna Room

Next steps: Pick colonies

Signature: \_\_\_\_\_

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

Name(s): sureb JodatDate and Time: 6/28/14 9:30 pmLiquid CultureAmount of LB used: 5 mL 8 tubesPart Used: the plating from page 228Antibiotic Used: AmpAmount of Antibiotic Used: 10 mLConcentration of Antibiotic: 100  $\mu$ g/mLPlates  
used

|                |   |
|----------------|---|
| L <sub>1</sub> | 10 <sup>-3</sup> , 10 <sup>-8</sup>                                       |
| L <sub>2</sub> | 10 <sup>-3</sup> , 10 <sup>-4</sup> , 10 <sup>-5</sup> , 10 <sup>-6</sup> |
| L <sub>3</sub> | 10 <sup>-3</sup> , 10 <sup>-4</sup>                                       |

Label on Product: ligation DH5 $\alpha$  w/ ligation productLocation of Product: warm room

Next Step: \_\_\_\_\_

~~500ml of 80% glycerol = 100ml~~

$\frac{250}{80} = 3.125$  factor  
 $3.125 \times 160 \text{ ml of } 40\% \text{ glycerol} = 500 \text{ } 50\% \text{ glycerol}$   
 $187.5 \text{ ml DepC water}$

Name(s): Jacob Jacob

Need 8 50...

$8 \times 3.125 = 2500 \text{ ml } 80\% \text{ glycerol}$

$8 \times 187.5 = 1500 \text{ ml DepC}$   
4000 ml total Glycerol stock  
Liquid Culture  
or 4 mL of 50% glycerol

Date and Time: 6/28/14 9:00pm

|                              |   |
|------------------------------|---|
| Amount of LB used:           | <u>500 ml liquid culture of DH5α cells w/ ligation products</u> |
| Part Used:                   | <u>500 ml 50% glycerol</u>                                      |
| Antibiotic Used:             | _____   |
| Amount of Antibiotic Used:   | _____   |
| Concentration of Antibiotic: | _____   |

|                      |   |
|----------------------|---|
| Label on Product:    | <u>DH5α w/ ligation products L# 10<sup>-8</sup></u> |
| Location of Product: | <u>Plasmid stock box -80°C iGEM 2013</u>            |
| Next Step:           | _____   |



Name(s): Jacob JodetDate and Time: 6/29/14 10:00pmLiquid CultureAmount of LB used: 5ml 8 tubesPart Used: dil another liquid culture from page 228electroporated  
DH5α cells w/ ligation products  
from page 227Antibiotic Used: AmpAmount of Antibiotic Used: 10 µLConcentration of Antibiotic: 100 µg/mL

| Ligation       | dilutions   |
|----------------|---|
| L <sub>1</sub> | 10 <sup>-3</sup> , 10 <sup>-4</sup>                                       |
| L <sub>2</sub> | 10 <sup>-3</sup> , 10 <sup>-4</sup> , 10 <sup>-5</sup> , 10 <sup>-6</sup> |
| L <sub>3</sub> | 10 <sup>-3</sup> , 10 <sup>-4</sup>                                       |

Label on Product: DH5α w/ ligation product L(##) 10<sup>-##</sup>Location of Product: warm room

Next Step: \_\_\_\_\_

## Zymo Mini Prep

Name(s): ANNA GARVEY + ~~REBECCA~~ MATT MORTENSENDate and Time: 6-30-14 11:00Check off as you  
complete the steps**Procedure:**Bacteria used: K1406000

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☒
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☒
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: K1406000

Location of product: \_\_\_\_\_

Deviations from Procedure and other Notes:

NEXT STEP:

Run GelContinued on back? Yes ☐; No ☒

## Gel Electrophoresis

Name: MATT MORRISON + ANNADate: 6-30-14

CARVEY

Time: 11:30am

% Agarose: \_\_\_\_\_

Lane Reagents

| Lane | Reagents |
|------|----------|
| 1    | 6        |
| 2    | 5        |
| 3    | 4        |
| 4    | 3        |
| 5    | 2        |
| 6    | 1        |
| 7    | 2 80034  |
| 8    | L        |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

# Gel Electrophoresis

Name:                       
Date:                       
Time:                     

Picture

Results

NEXT STEP:

## Gel Electrophoresis

Name: MATT MORTENSEN + ANNA  
Date: 7-6-30-14 GARLEY  
Time:       % Agarose: 1%

Lane Reagents

1

2

3

4

5

6

7

8

|

|

|

|

|

|

|

|

4

3

2

1

L

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture : No picture was taken before  
pieces of the gel were taken  
for a gel extraction

Results

NEXT STEP:

Gel Extraction

Date and Time: 6-30-14 Performed by: ANNA GARVEY + MATT MORTENSEN

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | 1      | 2    | 3    | 4    |  |  |  |  |  |
|-------------------|--------|------|------|------|--|--|--|--|--|
| Tube + Gel weight | 1.105  | 1.28 | 1.28 | 1.28 |  |  |  |  |  |
| Empty tube weight | .937   | .990 | .954 | .980 |  |  |  |  |  |
| Gel Weight        | .168   | .29  | .326 | .28  |  |  |  |  |  |
| Buffer QG         | .504ml | .87  | .978 | .84  |  |  |  |  |  |
| Isopropanol       | .168   | .29  | .326 | .28  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: K140600 Puri fied <P.235> <A/B>

Location of Product: Primer Box

Next Step:

Qubit + Electroporation

Date and Time: 7-1-14Performed by: ANNA GARVEY + MATT MORTENSEN**Qubit DNA Quantification****Master Mix:**

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

**Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

**Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

Name(s): Ana Garvey  
Matt Mortensen

## Electroporation

Page: 236 b

Reagents: 

### Cell Preparation

Competent cells thawed? Yes ☒ No ☐

Amount? 40  $\mu$ L

### Electroporation

Electroporation cuvette chilled? Yes ☐ No ☐

Amount of <sup>LB</sup> SOC added to culture tubes: 1 mL

DNA placed in m/f tubes? Yes ☒ No ☐

Placed on ice? Yes ☒ No ☐

Amount of DNA mixed with competent cells: 1  $\mu$ L

Voltage used for electroporation: ECR1

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No ☐

Amount added: 1 mL to 5 mL

Cells shaken at 37°C? Yes ☒ No ☐

Start time:            End time:           

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes ☒ No ☐

Cells spun in centrifuge (10-15s)? Yes ☒ No ☐

s/n decanted off? Yes ☒ No ☐

Cells resuspended? Yes ☒ No ☐

Amount of solution: 100  $\mu$ L

Dilutions performed:

10<sup>-1</sup> Yes ☒ No ☐

10<sup>-2</sup> Yes ☒ No ☐

10<sup>-3</sup> Yes ☒ No ☐

10<sup>-4</sup> Yes ☐ No ☐

10<sup>-5</sup> Yes ☐ No ☐

10<sup>-6</sup> Yes ☐ No ☐

10<sup>-7</sup> Yes ☐ No ☐

10<sup>-8</sup> Yes ☐ No ☐

10<sup>-9</sup> Yes ☐ No ☐

10<sup>-2</sup>             
10<sup>-4</sup>             
10<sup>-6</sup>           

Volumes: 100  $\mu$ L

Volumes: 100  $\mu$ L

Volumes: 100  $\mu$ L

Volumes:           

Volumes:           

Volumes:           

Volumes:           

Volumes:           

Volumes:           

Labels on products:           

Location of products:           

Next steps: check plates

Signature: 

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

Name(s): Zachary BimerDate and Time: 5:50 7/2/14Liquid CultureAmount of LB used: 5 ml x 3 tubesPart Used: K1406000Antibiotic Used: AmpAmount of Antibiotic Used: 10 ulConcentration of Antibiotic: 100 ug/mlLabel on Product: K1406000, 10°, Amp, 7/2/14, ZBLocation of Product: warm room, shakerNext Step: glycerol

Name(s): Zachary BirnerDate and Time: 12:30, 7/3/14~~Liquid Culture~~

Glycerol Stock

Amount of ~~LB~~ used: 500ml culture from 7/2/14, 500ml 40% glucose (bacteria)  
Part Used: K1406000 (Dr. Warner said to use it.)

Antibiotic Used: Amp

Amount of Antibiotic Used: \_\_\_\_\_

Concentration of Antibiotic: \_\_\_\_\_

Label on Product: K1406000, Amp<sup>R</sup>, 7/3/14, ZBLocation of Product: -80°C plasmid stock box iGEN RO13

Next Step: \_\_\_\_\_

Name(s): Ryan GeorgeDate and Time: 6h 7/8/14Liquid Culture

- |   |  |
|---|--|
| 1 | Amount of LB used: <u>5 mL x 4 cultures</u>                          |
| 2 | Part Used: <u>K1406000 - 800m - 80°F plasmid stock box isen 2013</u> |
| 3 | Antibiotic Used: <u>Amp</u>  |
| 4 | Amount of Antibiotic Used: <u>10 <math>\mu</math>L</u>               |
| 5 | Concentration of Antibiotic: <u>100 <math>\mu</math>g/mL</u>         |

1. 5 mL x 4 cultures
2. DH5 $\alpha$
3. N/A
4. N/A
5. N/A

Label on Product: on side of tubes - DH5 $\alpha$  - or - K1406000, RG, 7/8Location of Product: warm room shaker

Next Step: \_\_\_\_\_

## Zymo Mini Prep

Name(s): ANNA GARVEY

Date and Time: 7-9-14

Check off as you  
complete the steps**Procedure:**

Bacteria used: K1406000

1. Pellet bacteria via centrifuge at
- $\geq 8000$
- rpm for 3min. Discard supernatant.....

Note: This step differs from the printed instructions

2. Resuspend in 525
- $\mu$
- L of buffer sterile DI.....

Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L

3. Add 100
- $\mu$
- L 7X Lysis Buffer.....

Invert 4-6 times (less than 5 minutes until next step)

4. Add 350
- $\mu$
- L Neutralization Buffer.....

Inverting 4-6 times immediately

5. Centrifuge 6min at 13000rpm.....

6. Pipet supernatant into a QIAprep spin column.....

Centrifuge 30-60sec then discard flow through

Note: If multiple cultures of the same type are used, they are combined in this step

7. Add 200
- $\mu$
- L Endo-Wash Buffer to column.....

Centrifuge 30-60sec and then discard flow through

8. Add 400
- $\mu$
- L of Zyppy Wash Buffer to column.....

Centrifuge 30-60sec and then discard flow through

9. Transfer column to new microcentrifuge tube.....

10. Add 30
- $\mu$
- L Zyppy Elution Buffer to column.....

Let stand for 60sec then centrifuge for 60sec

11. Discard column and save centrifuge tube with flow through.....

12. Label and store in
- $-20^{\circ}\text{C}$
- for later use.....

Label on centrifuge tube: K1406000 Plasmid AG 7-9-14 C1-47

Location of product: PB Plasmid Box

Deviations from Procedure and other Notes:

NEXT STEP:

Qubit

Continued on back? Yes ☐; No ☒

Date and Time: 7-9-14 Performed by: ANNA GARVEY

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]



Date and Time: 7/9/14 1:00 pm Performed by: Matt Montenegro Anna Garvey

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer NE Buffer 2.1
- o DNA K1406000
- o Enzyme SpeI, PstI

| MATERIALS | TYPE             | AMOUNT USED            |
|-----------|------------------|------------------------|
| Buffer    | NE Buffer 2.1    | 5 $\mu$ L              |
| DNA       | K1406000 Plasmid | 2 $\mu$ g 8.06 $\mu$ L |
| Enzyme(s) | SpeI<br>PstI     | 2 $\mu$ L<br>2 $\mu$ L |
| Water     | DEPC             | 32.94 $\mu$ L          |

X 4

Total Reaction Volume

50  $\mu$ L

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 1:25 pm

End Time of Incubation: 1:25 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°

Method used for Quenching the Reaction (*different for different enzymes*)

80°C Heat Shock Inactivation for 20 min

Where did you store your finished product and what did you label it?

\_\_\_\_\_

NEXT STEP:

Date and Time: 7/9/14 3 pm Performed by: Matt Mortensen  
Anna Gervay

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer NE Buffer 2.1
- o DNA K1406000
- o Enzyme Xba I Pst I

| MATERIALS | TYPE             | AMOUNT USED            |
|-----------|------------------|------------------------|
| Buffer    | NEB 2.1          | 5 $\mu$ L              |
| DNA       | K1406000<br>p230 | 8.06                   |
| Enzyme(s) | X<br>P           | 2 $\mu$ L<br>2 $\mu$ L |
| Water     | DEPC             | 32.94                  |

Total Reaction Volume 50 $\mu$ L

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 3:15 pm

End Time of Incubation: 4:15 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

65°C Heat inactivation (20 min)

Where did you store your finished product and what did you label it?

\_\_\_\_\_

NEXT STEP:

# Gel Electrophoresis

Page <sup>4</sup>24

Name: Matt Martensen  
Anna Garvey  
 Date: 7-9-14  
 Time: 5:00pm

% Agarose: 1

Lane Reagents

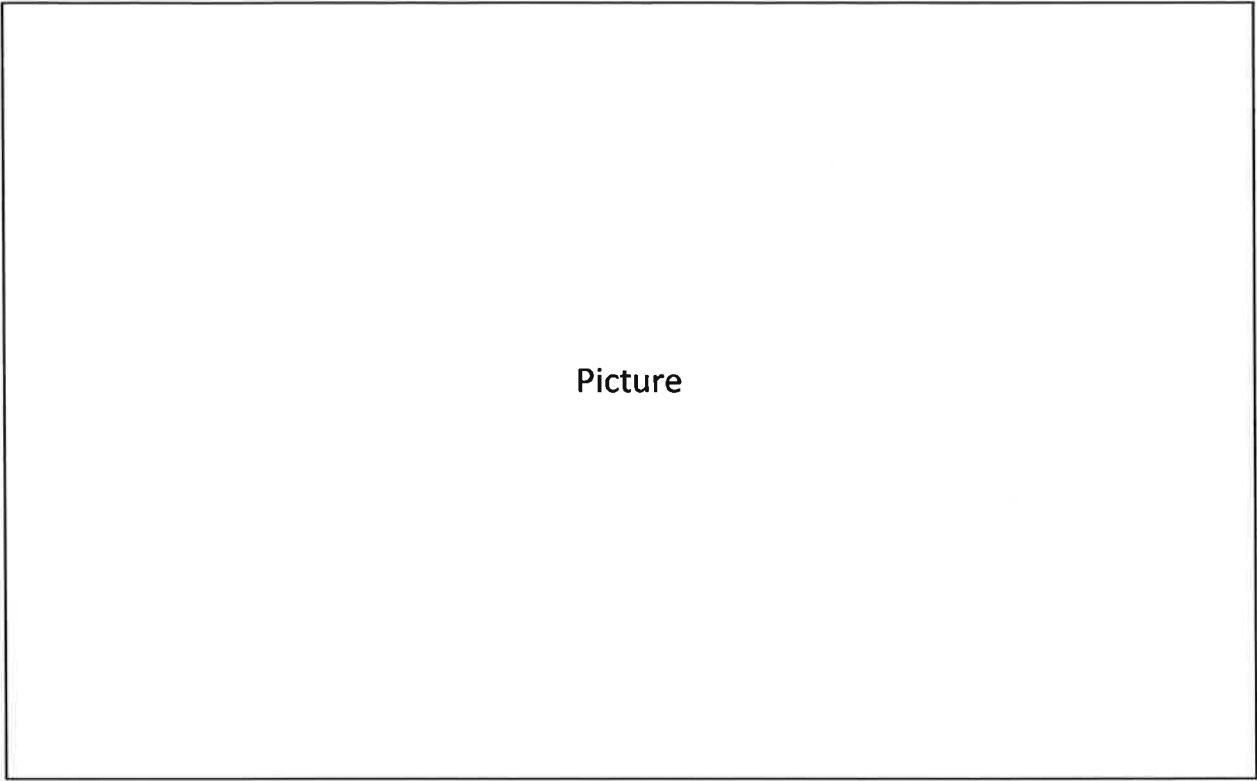
|   |               |                                    |  |
|---|---------------|------------------------------------|--|
| 1 | }             | K206000 Digest<br>Backbone (p.214) | <div>Reminders:</div> <div>Add Gel Green</div> <div>Add Loading Dye</div> <div>Run Red</div> |
| 2 |               |                                    |  |
| 3 |               |                                    |  |
| 4 | }             | K1406000 Digest (p.233)            |  |
| 5 |               |                                    |  |
| 6 |               |                                    |  |
| 7 | Uncut Plasmid |                                    |  |
| 8 | 1 Kb Ladder   |                                    |  |

Reagents used:

35 $\mu$ L DNA  
 6 $\mu$ L Loading Dye

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

NEXT STEP:

Date and Time: 7-9-14 6:50 Performed by: Matthew Montersen  
Anne Carver

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer NE Buffer 2.1
- o DNA K1406000
- o Enzyme Xba I, Pst I

| MATERIALS | TYPE                     | AMOUNT USED               |
|-----------|--------------------------|---------------------------|
| Buffer    | NEB 2.1                  | 2.5 $\mu$ L               |
| DNA       | K1406000<br>Plasmid p290 | 4.33 $\mu$ L<br>1 $\mu$ g |
| Enzyme(s) | Xba I<br>Pst I           | 1 $\mu$ L<br>1 $\mu$ L    |
| Water     | DEPC                     | 16.17 (+1 for Negatives)  |

Total Reaction Volume 25  $\mu$ L

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 0

End Time of Incubation: 0

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 80°C

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

NEXT STEP:



# Gel Electrophoresis

Page 246  
Name: MATTHEW MORTENSEN  
Date: ANNA GARVEY  
Time: 7-9-14  
8:55pm

% Agarose: 1%

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

2-10g Ladder  
- Pst I  
- Xba I  
Full  
1kb ladder

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

5μL DNA

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

## Zymo Mini Prep

Name(s): Matthew Mortenson Anna Garvey  
 Date and Time: 7-10-14 12 pm

Check off as you  
complete the steps

Procedure: 1K1406000 Amp x4

Bacteria used: ~~1K1406000~~

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☒
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☒
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☒
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: 1K1406000 Miniprep Amp p247  
 Location of product: Plasmid Box

Deviations from Procedure and other Notes:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 7/16/14 12:00pm Performed by: Daniel Schuer

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 6 | 2
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1300  $\mu$ L | 500  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 6.5  $\mu$ L | 2.5  $\mu$ L
- Subtract #3 from #2 1293.5  $\mu$ L | 497.5  $\mu$ L
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

# Results

| Sample Name                     | Page | Concentration         |
|---------------------------------|------|-----------------------|
| K215104 (Gel extract, R.D.) # b |      | 5.89 $\mu\text{g/mL}$ |
| K215104 (Gel extract, R.D.) # c |      | 2.10 $\mu\text{g/mL}$ |
| K215104 (Gel extract, R.D.) # d |      | 2.37 $\mu\text{g/mL}$ |
| J04450 ( " , " ) #1             |      | 2.93 $\mu\text{g/mL}$ |
| J04450 ( " , " ) #2             |      | 2.10 $\mu\text{g/mL}$ |
| J04450 ( " , " ) #3             |      | ~ (broken)            |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |
|                                 |      |                       |

Trial 1

Trial 2

3.65  $\mu\text{g/mL}$

2.09

2.29  $\mu\text{g/mL}$

2.56  $\mu\text{g/mL}$

2.29  $\mu\text{g/mL}$

\* ~~Qubit~~ Qubit had previously been performed on nanoprep ps. 198  
[lot higher concentration]

✓  
3

Date and Time: 7-10-14Performed by: ANNA GARVEY & MATTHEW MORTENSEN**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |         |  |  |  |  |  |  |  |  |
|-------------------|---------|--|--|--|--|--|--|--|--|
| Sample            | K206000 |  |  |  |  |  |  |  |  |
| Tube + Gel weight | 1.210   |  |  |  |  |  |  |  |  |
| Empty tube weight | .919    |  |  |  |  |  |  |  |  |
| Gel Weight        | .297    |  |  |  |  |  |  |  |  |
| Buffer QG         | .891    |  |  |  |  |  |  |  |  |
| Isopropanol       | .197    |  |  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.

6. Add 1 volume of isopropanol to each tube and mix gently.

7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)

10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: K206000 Gel Extraction 7-10-14 AG

Location of Product: Primer Box

Next Step:

Date and Time: 7-10-14 4:00pm Performed by: ANNA GARVEY

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer NEB Buffer 2.1
- o DNA K1406000
- o Enzyme XbaI, PstI

| MATERIALS | TYPE         | AMOUNT USED              |
|-----------|--------------|--------------------------|
| Buffer    | NEB 2.1      | 2.5ul                    |
| DNA       | K1406000     | 3.37ul                   |
| Enzyme(s) | XbaI<br>PstI | 1ul<br>1ul               |
| Water     | DEPC         | 17.13 (+1 for negatives) |

Total Reaction Volume 25ul

Construct a table with this format for each reaction that you do today.

$$\frac{267 \mu\text{g}}{\text{mL}} \div \frac{.297 \mu\text{g}}{1 \mu\text{L}} = 3.37 \mu\text{L}$$

~~11.13~~

Start Time of Incubation:

End Time of Incubation:

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 80°C

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

NEXT STEP:



Date and Time: 7-10-14 Performed by: Matt Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 4
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1300
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 1200/56.5
4. Subtract #3 from #2 1293.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

# Gel Electrophoresis

ANNA GARVEY Page 251  
Name: MATTHEW MORTENSEN  
Date: 7-10-14  
Time: 9:45pm

% Agarose: 1

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

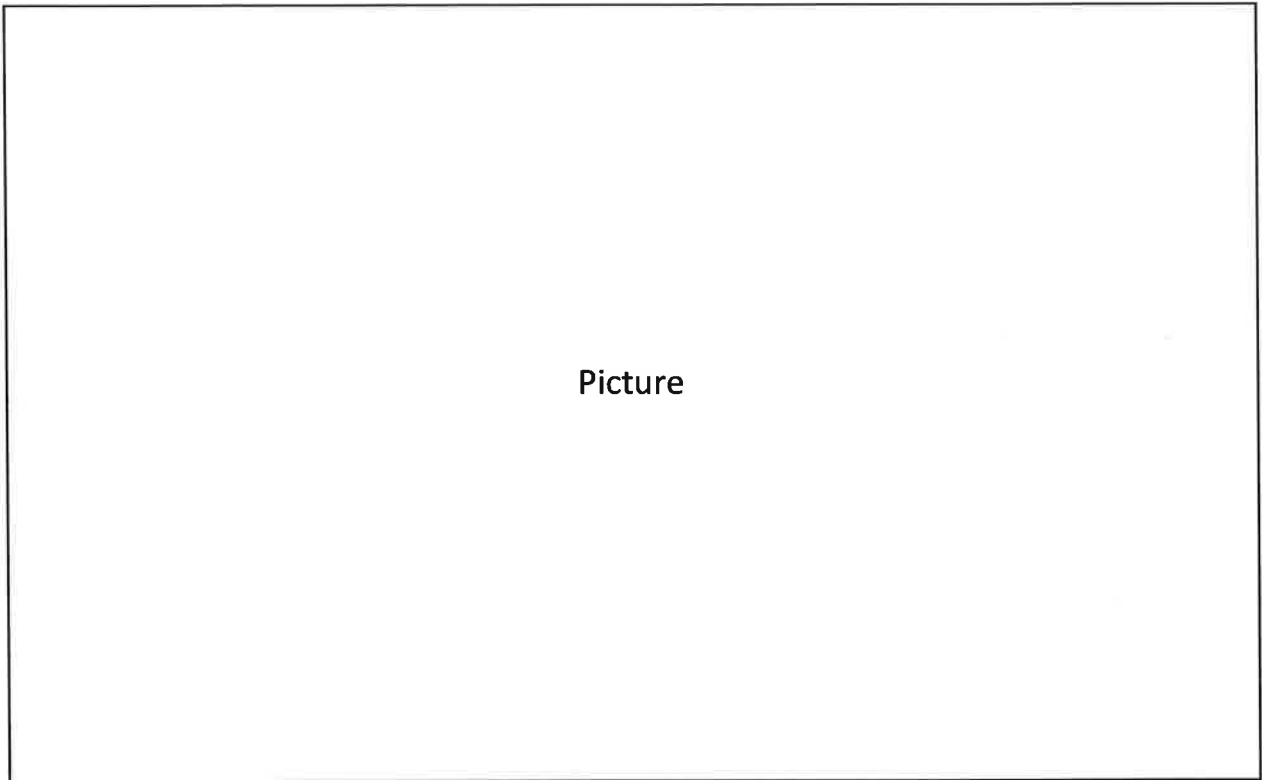
|  
|  
| - Pst I  
| - Xba I  
| Full Reaction (K1406000 + Pst I + Xba I)  
| Plasmid  
| 1 Kb Ladder  
|

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

|                   |
|-------------------|
| <p>NEXT STEP:</p> |
|-------------------|

Date and Time: 7-10-14  
Protocol: Lyophilization  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: MATT MORENSEN  
ANNA GARVEY  
DANIEL SCHROEDER

## Procedure (with applicable notes):

Combined 7 30ul Gel Extraction Elutions of J04450 and J025104 into individual mt tubes  
(~210ul totals)

The two samples were loaded into a Speed Vac SC110 Concentrator.  
Started at 3:10 on High drying rate (65°C)

Frequently changed ice

Came back to find dust/dirt residue in the tubes  
↳ Purified by resuspending in Elution Buffer and running through a spin column

## Results:

See Qubit

Location of product: \_\_\_\_\_  
Label on product: \_\_\_\_\_  
End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 7/11/14 8:15 AMPerformed by: David Schneider**Qubit DNA Quantification****Master Mix:**

1. Find out how many samples will need to be analyzed 2
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 900 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 4.5 $\mu$ L
4. Subtract #3 from #2 895.5 $\mu$ L
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

**Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

**Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

Results

| Sample Name   | Page | Concentration                      |
|---|------|------------------------------------|
| kz111011 (Gel extract) } continued after using<br>Johanna [.. ..] the Lypholization |      | too low for machine<br>too low " " |
|   |      |                                    |
|   |      |                                    |
|   |      |                                    |
|   |      |                                    |
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|   |      |                                    |
|   |      |                                    |
|   |      |                                    |
|   |      |                                    |

→ cannot perform Ligation = start over (A)

Name(s): Daniel S. [signature]

Date and Time: 7/11/14

Liquid Culture

Amount of LB used: 5 mL per tube; 30 mL total

Part Used: K215104; J04450

Antibiotic Used: Amp; Km

Amount of Antibiotic Used: 10 mL; 100 µg/mL

Concentration of Antibiotic: 100 µg/mL; 100 mg/mL

put in at 10:00 AM

Label on Product: Part; antibiotic; date; label

Location of Product: warm room

Next Step: next prep



## Zymo Mini Prep

Name(s): Daniel SchachDate and Time: 11:00pm 7/11/14Check off as you  
complete the steps**Procedure:**Bacteria used: K1215104 (A1) [unfortunately Jovase did not produce  
might have added in  
too much antibiotic]

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☐
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☐
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☐
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☐
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: mini prep 7/11/14  
K1215104 (A1)Location of product: Freezer if possible for  
separation team prep

Deviations from Procedure and other Notes:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Name(s): David Schmitt

Date and Time: 9/14/14 9:00pm

**Liquid Culture**

Amount of LB used: 5 mL per tube / 15 mL total

Part Used: Jen 26 → (CAU) glycerol stock

Antibiotic Used: kan *← says k3 which I imagine stands for kan*

Amount of Antibiotic Used: 3.5  $\mu$ L

Concentration of Antibiotic: 35  $\mu$ g/mL

Label on Product: part i antibiotic; date; location

Location of Product: room 200m

Next Step: ms prep

Name(s): Donald, David Zymo Mini Prep  
 Date and Time: 7:00 AM 7/15/14

*Follow instructions  
on ps. 255*

Check off as you  
complete the steps

**Procedure:**

Bacteria used: 70450 (A<sub>u</sub>)

1. Pellet bacteria via centrifuge at  $\geq 8000$  rpm for 3min. Discard supernatant. ☐
2. Resuspend in 250  $\mu$ L of buffer P1. ☐  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250 \mu$ L
3. Add 250  $\mu$ L Buffer P2. ☐  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350  $\mu$ L Buffer N3. ☐  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000 rpm. ☐
6. Pipet supernatant into a QIAprep spin column. ☐  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500  $\mu$ L Buffer PB to column. ☐  
 Centrifuge 30-60sec and then discard flow through
8. Add 750  $\mu$ L of Buffer PE to column. ☐  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube. ☐
10. Add 50  $\mu$ L Buffer EB to column. ☐  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through. ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use. ☐

Label on centrifuge tube: 70450 (A<sub>u</sub>)

Location of product: Freezer; section temp pump

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 11/15/14 12:00pm Performed by: David S. Green

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 6 + 2
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 400 + 100 = 500  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 4500 / 200 = 22.5  $\mu$ L
- Subtract #3 from #2 500 - 22.5 = 477.5  $\mu$ L
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

## Gel Electrophoresis

Name: Paul SchererDate: 7/13/14Time: 12:30 PM1:00 PM% Agarose: 1 %

Lane Reagents

1

2

3

4

5

6

7

8

Sambrook (A)  
M1000K255000 (A)  
M1000

Ladder

Reminders:

Add Gel Green

Add Loading Dye

Run Red

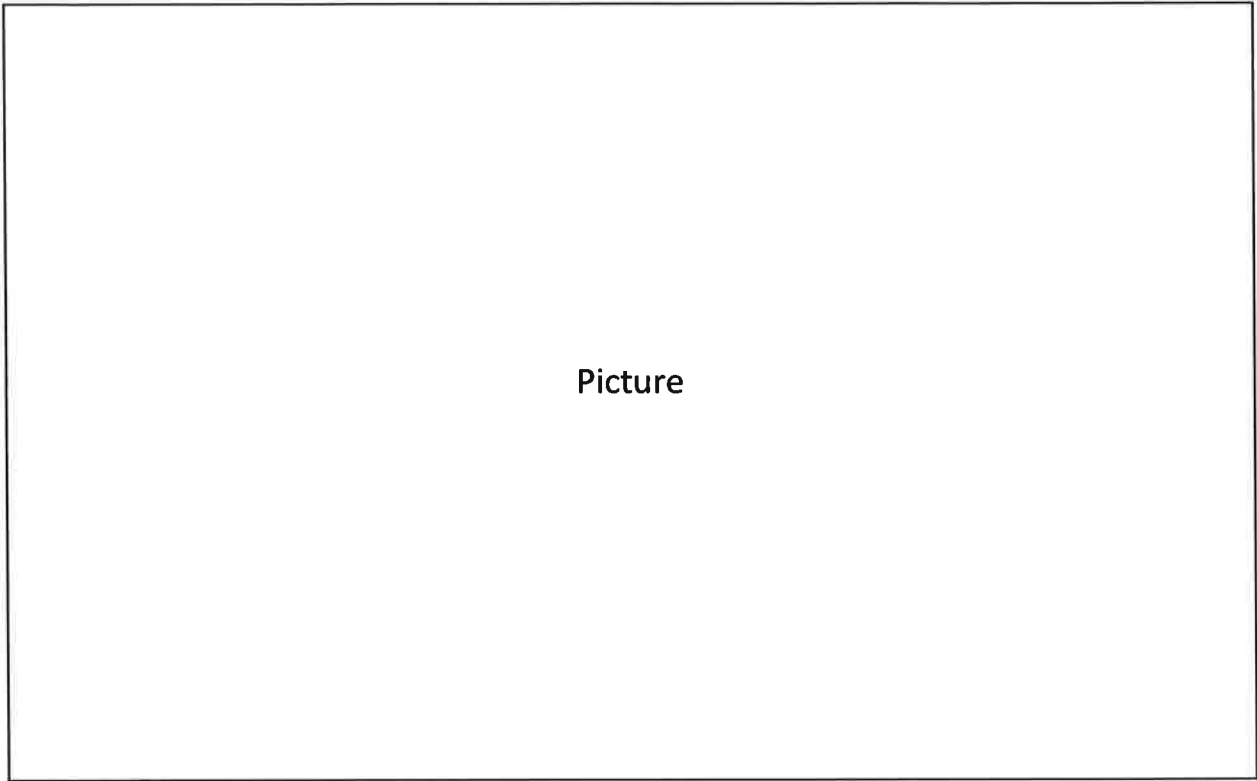
Reagents used:

1.5  $\mu$ l ladder  
 2.5  $\mu$ l sample + 1  $\mu$ l loading dye

Stored in slide door  
 fridge [I'm not sure  
 where U.V. light is]

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Picture

Results

NEXT STEP:

Date and Time: 7/15/14 4:45 Performed by: Zach Bimer**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB Buffer 2.1
- o DNA JO4450, K215104 [from miniprep 7/15/14]
- o Enzyme SpeI, EcoRI

| MATERIALS | TYPE          | AMOUNT USED      |
|-----------|---------------|------------------|
| Buffer    | NEB 2.1       | 2.5ml            |
| DNA       | JO4450        | 10ml             |
| Enzyme(s) | SPEI<br>EcoRI | 1.5 ml<br>1.5 ml |
| Water     | DEPC          | 9.5 ml           |

Total Reaction Volume 25ml

Construct a table with this format for each reaction that you do today.

|        |               |                  |
|--------|---------------|------------------|
| Buffer | NEB 2.1       | 2.5ml            |
| DNA    | K215104       | 10ml             |
| Enzyme | SpeI<br>EcoRI | 1.5 ml<br>1.5 ml |
| water  | DEPC          | 9.5 ml           |

25 total



Start Time of Incubation: 5:00 15

End Time of Incubation: 6:45

Note: incubation time is typically 1 hour <sup>was told to do 1 1/2 hrs</sup>

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (different for different enzymes)

Heat (20 min at 65°C)

Where did you store your finished product and what did you label it?

Digest (2B, 7/15)

A K or A J  
B K or B J  
C K or C J

K~~A~~ = K2B104  
J = J04450

placed in -20°C in secretory pump team box  
in PCR tubes

NEXT STEP: Gel

# Gel Electrophoresis

Page 261

Name: Zach Riner

Date: 7/16/14

Time: 1:15

% Agarose: 1%

Lane Reagents

1

| Ladder

2

| K21S104 A

3

| K21S104 B

4

| K21S104 C

5

| J04450 A

6

| J04450 B

7

| J04450 C

8

|

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

K21S104 digest

~~K21S~~

J04450 digest

6x loading dye

1kb Ladder

# Gel Electrophoresis

K215164 = 660+0  
Port = 4530

504450

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

Name(s): Zachary Birner  
  
Date and Time: 2:10 7/16/14Liquid CultureAmount of LB used: 5 ml x 12 tubes (60 total)Part Used: DH5aAntibiotic Used: NoneAmount of Antibiotic Used: NAConcentration of Antibiotic: NALabel on Product: DH5a, ZB, 7/16/14Location of Product: in warm room, on shaker, in metal rack of shakerNext Step:

Date and Time: 7/16/14 4:30 Performed by: Zach Birner

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | 1215104 A | 1215104 B | 1215104 C | 504450 A | 504450 B | 504450 C |  |  |  |
|-------------------|-----------|-----------|-----------|----------|----------|----------|--|--|--|
| Tube + Gel weight | 1.23      | 1.18      | 1.23      | 1.19     | 1.19     | 1.25     |  |  |  |
| Empty tube weight | .95       | .92       | .99       | .95      | .94      | .96g     |  |  |  |
| Gel Weight        | .28g      | .26g      | .24g      | .24g     | .25g     | .29g     |  |  |  |
| Buffer QG         | 840ul     | 780ul     | 720ul     | 720ul    | 750ul    | 870ul    |  |  |  |
| Isopropanol       | 280ul     | 260ul     | 240ul     | 240ul    | 250ul    | 290ul    |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: part, EB, extraction, for Bore, 7/16/14  
 Location of Product: secretion pump team box, -20°C  
 Next Step: ligation

Name(s): Jacob JodatDate and Time: 7/06/14 9:45pmLiquid CultureAmount of LB used: 5 mL x 3 culturesPart Used: K1406000 from -80°C plasmid stock box IBEM 2013 7/3/14Antibiotic Used: AmpAmount of Antibiotic Used: 10 mLConcentration of Antibiotic: 100 mg/mLLabel on Product: K1406000 date initialsLocation of Product: Warm room

Next Step: \_\_\_\_\_

## Zymo Mini Prep

Name(s): Matth Mortensen Anna GarveyDate and Time: ~~K1406000 (Amp)~~ 7-17-14 11:30 amCheck off as you  
complete the steps**Procedure:**Bacteria used: K1406000 (Amp) p230 x31. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒

Note: This step differs from the printed instructions

2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒

Invert 4-6 times (less than 5minutes until next step)

4. Add 350 $\mu$ L Neutralization Buffer..... ☒

Inverting 4-6 times immediately

5. Centrifuge 6min at 13000rpm..... ☒6. Pipet supernatant into a spin column..... ☒

Centrifuge 30-60sec then discard flow through

Note: If multiple cultures of the same type are used, they are combined in this step

7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒

Centrifuge 30-60sec and then discard flow through

8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☒

Centrifuge 30-60sec and then discard flow through

9. Transfer column to new microcentrifuge tube..... ☒10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☒

Let stand for 60sec then centrifuge for 60sec

11. Discard column and save centrifuge tube with flow through..... ☒12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒Label on centrifuge tube: K1406000 Amp 265Location of product: Plasmid Box

Deviations from Procedure and other Notes:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 7-17-14 Performed by: ANNA GARVEY

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

Date and Time: 12:30 7/17/14 Performed by: Daniel Schenck

## Qubit DNA Quantification

### Master Mix:

- Find out how many samples will need to be analyzed 6 samples | 2
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1340 $\mu$ L | 500 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 6.5 $\mu$ L | 2.5 $\mu$ L
- Subtract #3 from #2 1293.5 $\mu$ L | 497.5 $\mu$ L
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

### Standard and Sample Preparation:

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

| Sample Name             | Page | Concentration         |
|-------------------------|------|-----------------------|
| 204460 (A) Gel extract  |      | 14.2 $\mu\text{g/mL}$ |
| " (B) " "               |      | 15.7 $\mu\text{g/mL}$ |
| " (A) " "               |      | 18.5 $\mu\text{g/mL}$ |
| 2215104 (A) Gel extract |      | 10.5 $\mu\text{g/mL}$ |
| " (B) " "               |      | 7.42 $\mu\text{g/mL}$ |
| " (A) " "               |      | 8.09 $\mu\text{g/mL}$ |
|                         |      |                       |
|                         |      |                       |
|                         |      |                       |
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|                         |      |                       |
|                         |      |                       |
|                         |      |                       |
|                         |      |                       |

$$\frac{50}{14.2} = 3.52$$

$$\frac{50}{15.7} = 3.18$$

$$\frac{50}{18.5} = 2.7$$

for ligation

$$\frac{50}{10.5} = 4.76$$

$$\frac{50}{7.42} = 6.74$$

$$\frac{50}{8.09} = 6.18$$

Date and Time: 7/17/14 2:00 PM

Lab Technicians(s) involved: Daniel Schaub

Protocol: Ligation

Reagents:

Procedure (with applicable notes):

Control groups : Control A      Control B      Control C  
                          No Insert DNA      No DNA Ligase      No vector DNA  
                          No DNA Ligase

Gel extract  
 using (Q)

Reaction A      Reaction B      Reaction C      Insertion

|                      | A    | B    | C            | Control A | Control B    | Control C     |
|----------------------|------|------|--------------|-----------|--------------|---------------|
| DEPC water           | 8.12 | 7.08 | 8.72 $\mu$ L | 14.48     | 9.72 $\mu$ L | 12.24 $\mu$ L |
| T4 DNA Ligase        | 1    | 1    | 1 $\mu$ L    | —         | —            | 1 $\mu$ L     |
| T4 DNA Ligase Buffer | 2    | 2    | 2 $\mu$ L    | 2         | 2 $\mu$ L    | 2 $\mu$ L     |
| Vector DNA           | 2.7  | 3.18 | 3.52 $\mu$ L | 3.52      | 3.52 $\mu$ L | —             |
| Insert DNA           | 6.18 | 6.74 | 4.76 $\mu$ L | —         | 4.76 $\mu$ L | 4.76 $\mu$ L  |

Results:

Location of product: \_\_\_\_\_

Label on product: \_\_\_\_\_

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

Name(s): Daniel Schmitt

# Electroporation

Page: 269Reagents: LB

## Cell Preparation

Competent cells thawed? Yes ☒ No ☐Amount?                     

## Electroporation

Electroporation cuvette chilled? Yes ☒ No ☐Amount of SOC added to culture tubes: 1 mLDNA placed in m/f tubes? Yes ☒ No ☐Placed on ice? Yes ☒ No ☐Amount of DNA mixed with competent cells: 2  $\mu$ LVoltage used for electroporation: 2 kV

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No ☐Amount added: 1 mLCells shaken at 37°C? Yes ☒ No ☐Start time: 4:10 PM End time:                     

## Serial Dilution

Cells transferred to 1 mL m/f tube? Yes ☐ No ☐Cells spun in centrifuge (10-15s)? Yes ☐ No ☐s/n decanted off? Yes ☐ No ☐Cells resuspended? Yes ☐ No ☐Amount of solution:                     

Dilutions performed:

 $10^{-1}$  Yes ☐ No ☐Volumes:                      $10^{-2}$  Yes ☐ No ☐Volumes:                      $10^{-3}$  Yes ☐ No ☐Volumes:                      $10^{-4}$  Yes ☐ No ☐Volumes:                      $10^{-5}$  Yes ☐ No ☐Volumes:                      $10^{-6}$  Yes ☐ No ☐Volumes:                      $10^{-7}$  Yes ☐ No ☐Volumes:                      $10^{-8}$  Yes ☐ No ☐Volumes:                      $10^{-9}$  Yes ☐ No ☐Volumes:                     Labels on products:                     Location of products:                     Next steps:                     Signature:                     From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

# Gel Electrophoresis

Page <sup>270</sup>~~267~~

Name: Matt Mortensen Anna Garvey

Date: 7-17-14

Time: 12:40pm

% Agarose: 1

Lane Reagents

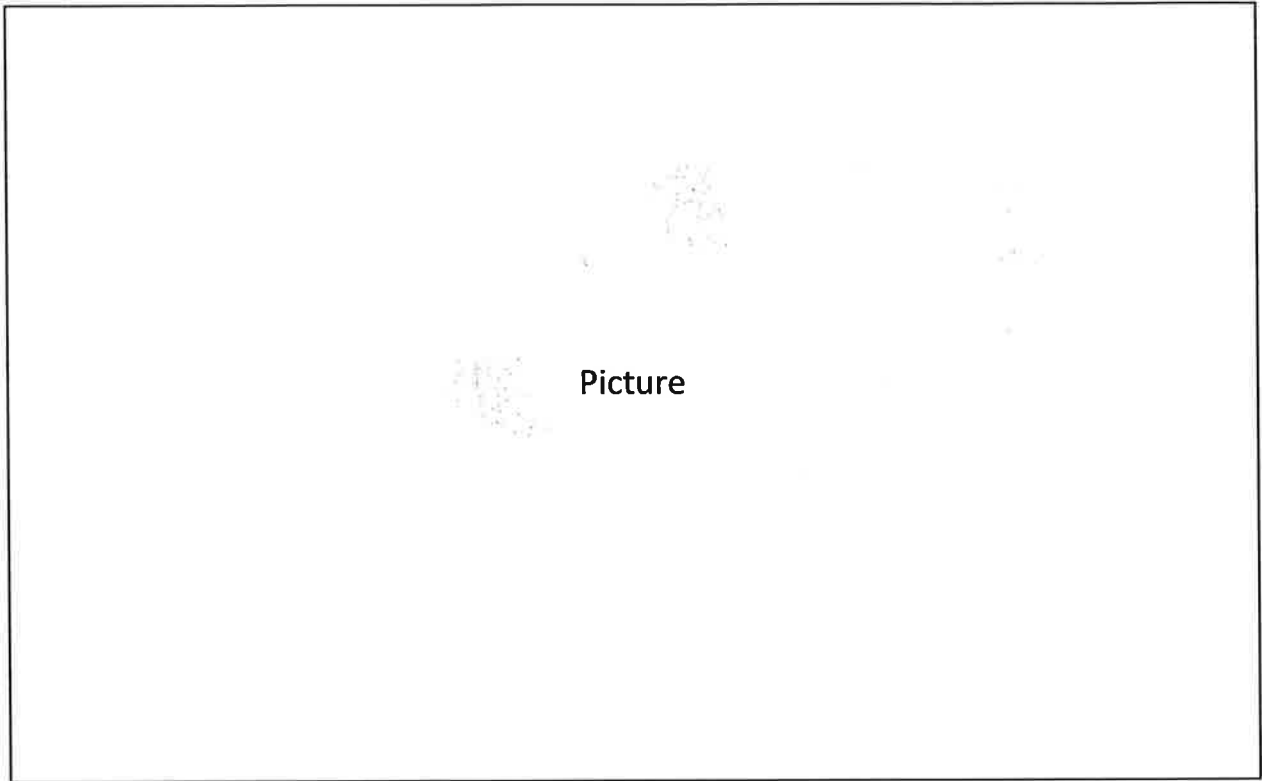
|   |                                    |
|---|------------------------------------|
| 1 | 1kb ladder                         |
| 2 | p 265 K1406000 Miniprep Product #1 |
| 3 | p 265 K1406000 Miniprep Product #2 |
| 4 | p 265 K1406000 Miniprep Product #2 |
| 5 | p 247 K1406000 Miniprep Product    |
| 6 | p 212 B0034 Miniprep Product       |
| 7 |                                    |
| 8 |                                    |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

NEXT STEP:

Date and Time: 7/17/14 4pm Performed by: Matt Mjstenen  
Anna Garvey

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer \_\_\_\_\_
- o DNA \_\_\_\_\_
- o Enzyme \_\_\_\_\_

| MATERIALS | TYPE                     | AMOUNT USED |
|-----------|--------------------------|-------------|
| Buffer    | NEB 2.1                  | 2.5         |
| DNA       | BOOZY A p212<br>1ug MB6K | 11.56uL     |
| Enzyme(s) | SpeI<br>PstI             | 1uL<br>1uL  |
| Water     | DEPC                     | 8.94        |

Negative

2.5uL

5.78uL

Total Reaction Volume 25uL

.5uL or 0uL

0uL .5uL

16.22

Construct a table with this format for each reaction that you do today.

|        |                       |            |                         |                               |
|--------|-----------------------|------------|-------------------------|-------------------------------|
| Buffer | NEB 2.1               | 5uL        | Negative<br>2.5uL       |                               |
| DNA    | K215104 A p212<br>2ug | 9.0uL      | 2.26uL                  |                               |
| Enzyme | XbaI<br>PstI          | 2uL<br>2uL | .5uL or 0uL<br>0uL .5uL | 50uL Normal<br>25uL Negatives |
| Water  | DEPC                  | 31.95      | 19.74uL                 |                               |



Start Time of Incubation: 4:20 pm

End Time of Incubation: 5:20 pm

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

80°C Heat Inactivation for 20 min

Where did you store your finished product and what did you label it?

NEXT STEP:

# Gel Electrophoresis

Page 272

Name: Matthew Mortensen  
Anna Gervais

Date: 7/17/14

Time: 6 pm

% Agarose: 1

Lane Reagents

|   |                                |      |
|---|--------------------------------|------|
| 1 | 1 kb ladder                    | 24L  |
| 2 | K215104 Digest                 | 40uL |
| 3 | K215104 Digest                 | 40uL |
| 4 | K215104 Digest - PstI Negative | 25uL |
| 5 | K215104 Digest - XbaI Negative | 25uL |
| 6 | B0034 Digest                   | 25uL |
| 7 | B0034 Digest - PstI Negative   | 25uL |
| 8 | B0034 Digest - SpeI Negative   | 25uL |

} p271

Reminders:

Add Gel Green

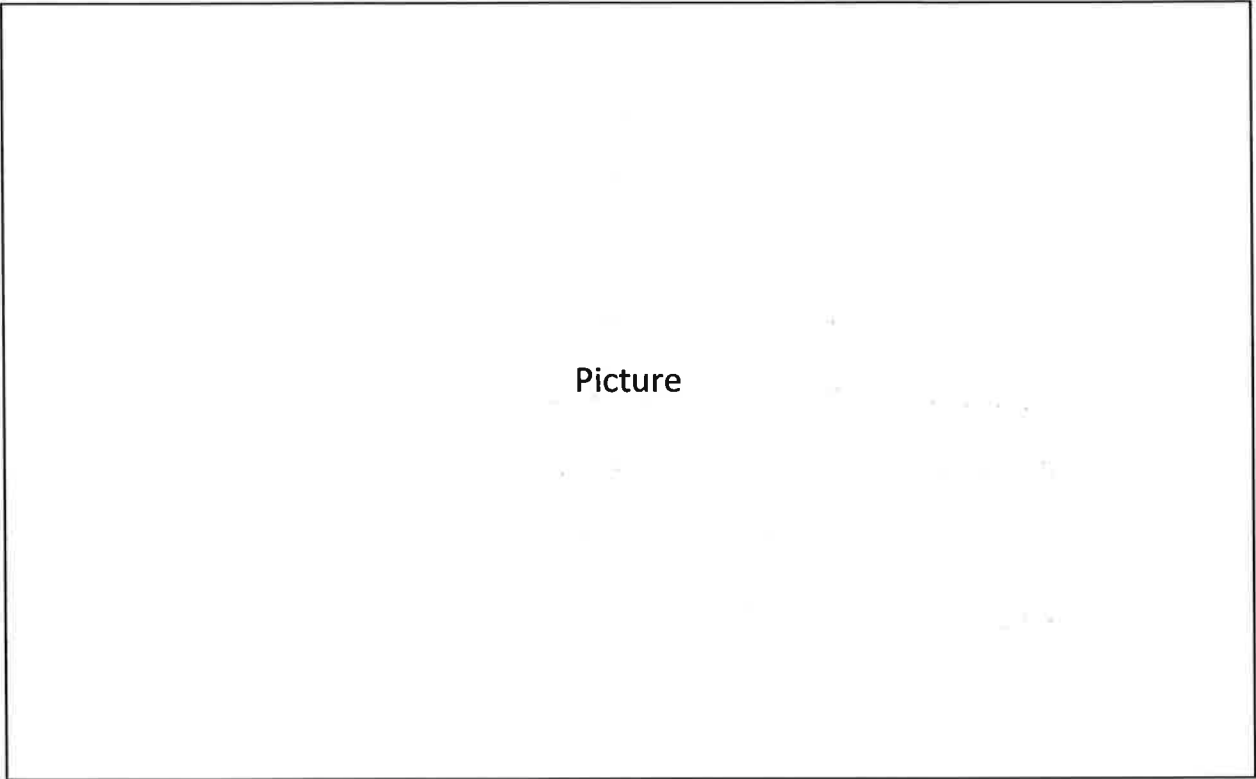
Add Loading Dye

Run Red

Reagents used:

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

|            |
|------------|
| NEXT STEP: |
|------------|

## Zymo Mini Prep

Name(s): Matt Mortensen Christa WinslowDate and Time: 8/19/14 7:00 pmCheck off as you  
complete the steps**Procedure:**K314200: psBIC3 p-cumate operon  
K875001: psBIC3 Tse2 ToxinBacteria used: K875004: psBIC3 Adenovirus particle (backbone for SOEing PCR ligation) x2

1. Pellet bacteria via centrifuge at
- $\geq 8000$
- rpm for 3min. Discard supernatant.....

Note: This step differs from the printed instructions

2. Resuspend in 525
- $\mu$
- L of buffer sterile DI.....

Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L

3. Add 100
- $\mu$
- L 7X Lysis Buffer.....

Invert 4-6 times (less than 5 minutes until next step)

4. Add 350
- $\mu$
- L Neutralization Buffer.....

Inverting 4-6 times immediately

5. Centrifuge 6min at 13000rpm.....

6. Pipet supernatant into a spin column.....

Centrifuge 30-60sec then discard flow through

Note: If multiple cultures of the same type are used, they are combined in this step

7. Add 200
- $\mu$
- L Endo-Wash Buffer to column.....

Centrifuge 30-60sec and then discard flow through

8. Add 400
- $\mu$
- L of Zippy Wash Buffer to column.....

Centrifuge 30-60sec and then discard flow through

9. Transfer column to new microcentrifuge tube.....

10. Add 30
- $\mu$
- L Zippy Elution Buffer to column.....

Let stand for 60sec then centrifuge for 60sec

11. Discard column and save centrifuge tube with flow through.....

12. Label and store in
- $-20^{\circ}\text{C}$
- for later use.....

Label on centrifuge tube: (Part) 2 Backbone Plasmid 8/19 MWMLocation of product: Plasmid Box (p273)

Deviations from Procedure and other Notes:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 8/19/14 Performed by: Christa Winslow

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed 4
  - Note: You will need enough master mix for two more (The standards) 6 total
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1300 $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ~~650~~ 6.5 $\mu$ L
4. Subtract #3 from #2 ~~650~~ 1293.5
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

## PCR

Date and Time: 8-20-14Performed by: ANNA GARVEY

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer MidSci Bullsseye 2x PCR MASTER MixdNTPs MMForward Primer Safety Primer AReverse Primer Safety Primer BTemplate DNA KB75001Taq DNA Polymerase MMNuclease-free water DepC

Procedure:

| Symbol on lid  | Content in PCR tube   |
|----------------|---|
| AB             | 12.5 $\mu$ L MM + 1.25 $\mu$ L Primer A + 1.25 $\mu$ L B + 5 $\mu$ L B75001 + 5 $\mu$ L DepC  |
| CD             | <del>12.5<math>\mu</math>L MM + 1.25<math>\mu</math>L Primer C + 1.25<math>\mu</math>L Primer D + 5<math>\mu</math>L B15200 + 5<math>\mu</math>L DepC</del> |
| N <sub>1</sub> | No B75001 $\rightarrow$ 10 $\mu$ L DepC   |
| N <sub>2</sub> | No B15200 $\rightarrow$ 10 $\mu$ L DepC   |
| N <sub>3</sub> | No Primers AB $\rightarrow$ 7.5 DepC  |
| N <sub>4</sub> | No Primers CD $\rightarrow$ 7.5 DepC  |
| N <sub>5</sub> | No MM $\rightarrow$ 17.5 DepC   |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 55°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) \_\_\_\_\_

End notes/comments:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: 8-20-14

Performed by: Matthew Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 4
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1300
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200  $\searrow$
4. Subtract #3 from #2  $\searrow$
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

## PCR

Date and Time: 8-20-14 Performed by: ANNA GARVEY

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer MidSci MasterMix 2xdNTPs MMForward Primer C PrimerReverse Primer D PrimerTemplate DNA 314200Taq DNA Polymerase MMNuclease-free water DepC

## Procedure:

| Symbol on lid  | Content in PCR tube   |
|----------------|---|
| CD             | 12.5MM + 1.25 C + 1.25D + 5 $\mu$ L 315200 + 5 $\mu$ L DepC |
| N <sub>1</sub> | No 315200 $\rightarrow$ 10 $\mu$ L DepC                     |
| N <sub>2</sub> | No Primers CD $\rightarrow$ 7.5 $\mu$ L DepC                |
| N <sub>3</sub> | No MM $\rightarrow$ 17.5 DepC                               |
|                |   |
|                |   |
|                |   |

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 61°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) \_\_\_\_\_

End notes/comments:

## NEXT STEP:

Continued on back? Yes ☐; No ☐

Name(s): Matthew MorkenDate and Time: 8-28<sup>3</sup>-14 8pmLiquid CultureAmount of LB used: 5 mLPart Used: K215002 (plac + RBS + Tag) (B2 in Box)Antibiotic Used: AmpAmount of Antibiotic Used: 5  $\mu$ LConcentration of Antibiotic: 50  $\mu$ g/mLLabel on Product: K215002 Amp MM 8-28-14Location of Product: Warm RoomNext Step: Mini prep

## Gel Electrophoresis

Name: Matt MurthaDate: 8-23-14Time: 8:30% Agarose: 1%

Lane Reagents

|   |                   |                                      |   |
|---|-------------------|--------------------------------------|---|
| 1 | L                 | 1kb NEB DNA ladder                   | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | AB                | p <sup>275</sup> KS75001 PCR Product |   |
| 3 | AB N <sub>1</sub> | p <sup>275</sup>                     |   |
| 4 | AB N <sub>2</sub> | p <sup>275</sup>                     |   |
| 5 | CD                | p <sup>277</sup> K314200 PCR Product |   |
| 6 | CD N <sub>1</sub> | p <sup>277</sup>                     |   |
| 7 | CD N <sub>2</sub> | p <sup>277</sup>                     |   |
| 8 |                   |                                      |   |

Reagents used: 3  $\mu$ L of each DNA sample 1  $\mu$ L 1 kb Ladder

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results AB W<sub>1</sub> has extra DNA farther from the well?  
over all it looks like AB failed  
CD looks good.

NEXT STEP:

## Zymo Mini Prep

Name(s): Matt Mortenson  
 Date and Time: 8-24-14 10 am

Check off as you  
complete the steps

**Procedure:**

Bacteria used: K215002 Amp (freezer stock #2)

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
- Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☐
- Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☐
- Invert 4-6 times (less than 5minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☐
- Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☐
6. Pipet supernatant into a spin column..... ☐
- Centrifuge 30-60sec then discard flow through
- Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zippy Wash Buffer to column..... ☐
- Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 30 $\mu$ L Zippy Elution Buffer to column..... ☐
- Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: K215002 Plasmid Amp MWM p280

Location of product: Plasmid Box

Deviations from Procedure and other Notes:

NEXT STEP:

Continued on back? Yes ☐; No ☐

Mini Prep - ~~D~~ New Protocol

Name(s): ANNA GARNEY  
Date and Time: 8-26-14 @ 9:30am

Check off as you  
complete the steps

**Procedure:**

Bacteria used: KB75001

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
2. Resuspend in 250 $\mu$ L of buffer P1..... ☐  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☐  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☐  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☐  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☐  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 50 $\mu$ L Buffer EB to column..... ☐  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: KB75001 Chlam AG 8-26-14

Location of product: Plasmid Box

Deviations from Procedure and other Notes:

Successful?

yes

NEXT STEP:

AB PCR

Continued on back? Yes ☐; No ☐

Date and Time: 8-26-14 @ 10am Performed by: ANNA GARVEY

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed     
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L     
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

## PCR

Date and Time: 8/25/14 3:50 pm

Performed by: Anna Carvey  
Matt Mortensen

Reagents: (customize the list and include volumes and concentration):

|   |                            |         |
|---|----------------------------|---------|
| ThermoPol or Standard Taq Reaction Buffer | Bullseye 2x MasterMix      | 12.5 uL |
| dNTPs                                     |                            | 1.25 uL |
| Forward Primer                            | SOEing Primer A            | 1.25 uL |
| Reverse Primer                            | SOEing Primer B            | 1.25 uL |
| Template DNA                              | 187501 Plasmid p281 (1 ug) | 7.46 uL |
| Taq DNA Polymerase                        |                            |         |
| Nuclease-free water                       | DepC                       | 2.54 uL |

## Procedure:

|  |
|--|
|  |
|--|

| Symbol on lid  | Content in PCR tube         |
|----------------|-----------------------------|
| AB             | Full Reaction               |
| N <sub>1</sub> | No Template 10 uL DepC      |
| N <sub>2</sub> | No Primers 5.04 uL DepC     |
| N <sub>3</sub> | No Master Mix 15.04 uL DepC |
|                |                             |
|                |                             |
|                |                             |

x2

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 63°C |
| Extension    | 72°C |
| Final        | 72°C |

Number of cycles: 34

Time of completion: \_\_\_\_\_

Label on product(s): \_\_\_\_\_

Location of product(s) \_\_\_\_\_

End notes/comments:

|  |
|--|
|  |
|--|

## NEXT STEP:

|  |
|--|
|  |
|--|

Continued on back? Yes ☐; No ☐

Date and Time: 8-29-14 Performed by: Matt Mortensen

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed \_\_\_\_
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_
4. Subtract #3 from #2 \_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

| Sample Name     | Page | Concentration             |
|-----------------|------|---------------------------|
| AB <sub>1</sub> | 283  | <del>3.48</del> 27.0 ug/L |
| AB <sub>2</sub> | 283  | 17.5 ug/L                 |
| N <sub>1</sub>  | 283  | 4.17 ug/L                 |
| N <sub>2</sub>  | 283  | —                         |
| N <sub>3</sub>  | 283  | —                         |
| CD              | 277  | 23.4 ug/L                 |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |
|                 |      |                           |

PCR seems to have been successful

Sample  
CD  
N<sub>1</sub>  
N<sub>2</sub>  
N<sub>3</sub>

Page  
277

Concentration  
23.4

sewing

PCR

Date and Time: 7:30 8/27/14

Performed by: Jacob Sedat

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer Bullseye 2X PCR master mix 12.5  $\mu$ l  
dNTPs in mMForward Primer 1.25  $\mu$ l A primerReverse Primer 1.25  $\mu$ l B primerTemplate DNA 5  $\mu$ l K314200, 2  $\mu$ l K875001  $\rightarrow$  AB pg. 283Taq DNA Polymerase in mM  $\rightarrow$  CD pg. 277

Nuclease-free water Dep C

25  $\mu$ l rxn

Procedure:

did not have enough CD, so E and F only received 1  $\mu$ l of CD

| Symbol on lid | Content in PCR tube  |
|---------------|--|
| A             | 2 $\mu$ l Dep C, 5 $\mu$ l K314200, 2 $\mu$ l K875001, 12.5 $\mu$ l MM, 1.25 $\mu$ l A primer, 1.25 $\mu$ l B primer |
| B             | " " 12.5 $\mu$ l MM, 8.5 $\mu$ l Dep C   |
| C             | 1 $\mu$ l K314200 " 1.25 $\mu$ l A primer, 1.25 $\mu$ l B primer, 1.25 $\mu$ l Dep C                                 |
| D             | 10 $\mu$ l Dep C, 1.25 $\mu$ l A primer, 1.25 $\mu$ l B primer, 12.5 $\mu$ l MM                                      |
| E             | 1 $\mu$ l K314200, 1 $\mu$ l Dep C, 1.25 $\mu$ l A primer, 1.25 $\mu$ l B primer, 12.5 $\mu$ l MM                    |
| F             | 8 $\mu$ l Dep C, 2 $\mu$ l K875001, 1.25 $\mu$ l A primer, 1.25 $\mu$ l B primer, 12.5 $\mu$ l MM                    |

Positive  
no primers  
no MM  
no template  
no K875001  
no K314200

## Temperature Settings

|              |      |
|--------------|------|
| Denaturation | 95°C |
| Annealing    | 62°C |
| Extension    | 72°C |
| Final        | 4°C  |

Number of cycles: 34

Time of completion: 10:21 pm

Label on product(s): A, C, D, E, F

JS 8/27/14

Location of product(s) in 20 on green holder

End notes/comments:

Successful

NEXT STEP:

Continued on back? Yes ☐; No ☐

Jacob Todat

Date and Time: 8/27/14 10:30 pm Performed by: Matt Mortensen Anna Garvey**Qubit DNA Quantification****Master Mix:**

- Find out how many samples will need to be analyzed 6
  - Note: You will need enough master mix for two more (The standards)
- Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L 1700  $\mu$ L
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
- Take #2 and divide it by 200 9.5
- Subtract #3 from #2 1691.5
- Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
- Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
- Add #3 of 200X Quant-iT dsDNA BR Reagent
- Vortex lightly to mix.

**Standard and Sample Preparation:**

- In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
- Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
- For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

**Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

- At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
- Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
- If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
- Repeat step #3 for each sample
- Once all of your samples have been run, simply discard the tubes.

## Results

| Sample Name | Page | Concentration         |
|-------------|------|-----------------------|
| A           | 285  | 37.7 $\mu\text{g/mL}$ |
| B           | "    | 118 $\mu\text{g/mL}$  |
| C           | "    |                       |
| D           | "    | 377 $\mu\text{g/mL}$  |
| E           | "    | 38.0 $\mu\text{g/mL}$ |
| F           | "    | 12.3 $\mu\text{g/mL}$ |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |
|             |      |                       |

Redo qubit, vortex next time  
negative D should be zero

Date and Time: \_\_\_\_\_ Performed by: \_\_\_\_\_

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed \_\_\_\_\_
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_\_
4. Subtract #3 from #2 \_\_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.



## Results

[illegible]

Date and Time: 8/29/14 Performed by: Matt Mortensen Anna Garvey**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

|                   |             |  |  |  |  |  |  |  |  |
|-------------------|-------------|--|--|--|--|--|--|--|--|
| Sample            | SUEing Prod |  |  |  |  |  |  |  |  |
| Tube + Gel weight | 1.093g      |  |  |  |  |  |  |  |  |
| Empty tube weight | 0.938g      |  |  |  |  |  |  |  |  |
| Gel Weight        | .155g       |  |  |  |  |  |  |  |  |
| Buffer QG         | 465 $\mu$ L |  |  |  |  |  |  |  |  |
| Isopropanol       | 155 $\mu$ L |  |  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.

6. Add 1 volume of isopropanol to each tube and mix gently.

7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)

10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: 286 Sewing PCR ExtractLocation of Product: Primer Box

Next Step:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: 1

Lane Reagents

1

F

2

E

3

D

4

C

5

B

6

A

7

~~A~~

8

L

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

The gel looks successful.

NEXT STEP:

Gel Extract

Date and Time: \_\_\_\_\_

Performed by: Matt Moresen & Anna Garvey**Qubit DNA Quantification****Master Mix:**

1. Find out how many samples will need to be analyzed \_\_\_\_
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_
4. Subtract #3 from #2 \_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

**Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

**Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

## PCR

Date and Time: 8/28/14

Performed by: Matz Mortensen

Reagents: (customize the list and include volumes and concentration):

ThermoPol or Standard Taq Reaction Buffer 12.5  $\mu$ l 2X Bulldog's Master Mix

dNTPs

 $\mu\mu$ 

## Forward Primer

1.25  $\mu$ L SOEing "A" primer

Reverse Primer

1.25  $\mu$ l 50 "D" primer

## Template DNA

2  $\mu$ L 50Eing PCR Product p 285 "B"

Taq DNA Polymerase

mm

Nuclease-free water

8uL

**Procedure:**

\_\_\_\_\_

[illegible]

| Temperature Settings |    |
|----------------------|----|
| Denaturation         | 95 |
| Annealing            | 60 |
| Extension            | 72 |
| Final                | 72 |

Number of cycles: 50

Time of completion: \_\_\_\_\_

Label on product(s):

Location of product(s)

End notes/comments:

\_\_\_\_\_

**NEXT STEP:**

\_\_\_\_\_

Continued on back? Yes ☐; No ☐

Date and Time: 8-28-14 Performed by: ANNA GARVEY + MATT MORTENSEN

### Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

### PROCEDURE

#### Material Location

- o Buffer 2.5 NEB 2.1
- o DNA 20.83 K875004
- o Enzyme 0.5 0.5 EcoRI, XbaI

| MATERIALS | TYPE                                   | AMOUNT USED |
|-----------|--|-------------|
| Buffer    | NEB 2.1                                | 2.5 ul      |
| DNA       | <del>K875004</del> <sup>SEWINGRR</sup> | 20.83ul     |
| Enzyme(s) | EcoRI<br>XbaI                          | 0.5ul       |
| Water     | DEPC                                   | 0.77ul      |

Total Reaction Volume 25

64 ug/mL

Construct a table with this format for each reaction that you do today.

K875004 7.81ul  
 Buffer 2.5ul  
 Enzymes 0.5ul  
 Water 14.2 ~~(14.2)~~  
 N1 = 4.9 <sup>plus</sup> template  
       ~~2.5~~ buffer  
       2x 0.5 enzyme  
       ~~14.2~~ DepC  
       ~~19.0~~ 20.1  
 N2 = 4.9 template  
       2.5 buffer  
       21.6 ul DepC



Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

NEXT STEP:

## Gel Electrophoresis

Name: ANNA GARVEY + MAH MOHAMED

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: 1

Lane Reagents

|   |                    |
|---|--------------------|
| 1 |                    |
| 2 |                    |
| 3 |                    |
| 4 | N2 (-enzymes)      |
| 5 | N1 (-buffer)       |
| 6 | B (KB75004 Digest) |
| 7 | A (SEW PCR Digest) |
| 8 | Ladder             |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

The gel was successful

NEXT STEP:

Gel Extraction

Date and Time: \_\_\_\_\_ Performed by: Matthew Mortensen**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

From p 291

| Sample            | PCR         | Vector      |  |  |  |  |  |  |  |
|-------------------|-------------|-------------|--|--|--|--|--|--|--|
| Tube + Gel weight | 1.075       | 1.150       |  |  |  |  |  |  |  |
| Empty tube weight | .914 g      | .943 g      |  |  |  |  |  |  |  |
| Gel Weight        | .161 g      | .207        |  |  |  |  |  |  |  |
| Buffer QG         | 483 $\mu$ L | 621 $\mu$ L |  |  |  |  |  |  |  |
| Isopropanol       | 161 $\mu$ L | 207 $\mu$ L |  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.

6. Add 1 volume of isopropanol to each tube and mix gently.

7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)

10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product:

Location of Product:

Next Step:

Date and Time: 8/30/14

Performed by: Matt Merton

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 2
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_
4. Subtract #3 from #2 \_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: \_\_\_\_\_ Performed by: ANNA Canvey + Matt MORTENSEN**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer 2.5 NEB 2.1
- o DNA ~~1000000~~ KB75004, Sew PCR
- o Enzyme EcoRI, XbaI, SpeI ~~1000000~~

| MATERIALS | TYPE                          | AMOUNT USED             |
|-----------|-------------------------------|-------------------------|
| Buffer    | NEB 2.1                       | 2.5 $\mu$ L             |
| DNA       | sewing PCR (p289)             | 4.83 $\mu$ L            |
| Enzyme(s) | EcoRI<br><del>XbaI</del> SpeI | <del>1000000</del> (x2) |
| Water     | DEPC                          | 15.07                   |

Total Reaction Volume 25  $\mu$ L

Construct a table with this format for each reaction that you do today.

(273P)  
 KB75004 7.81  $\mu$ L  
 Buffer 2.5  $\mu$ L  
 Enzymes (Ex) 1  $\mu$ L  
 Water 13.2  $\mu$ L

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

20min at 65°C

Where did you store your finished product and what did you label it?

Plasmid Box

NEXT STEP:



# Gel Electrophoresis

Page 294b

Name: Matthew Mortensen

Date: 8-31-14

Time: 5pm

% Agarose: 1

Lane Reagents

1  
2  
3  
4  
5  
6  
7  
8

|   |  |
|---|--|
|   |  |
| 1 kb NEB DNA Ladder   |  |
| SOEing PCR Product Digest p294 40ul                                     |  |
| <del>KB75004 Plasmid Digest p294 25ul</del> SOEing PCR Prod Digest p294 |  |
| KB75004 Digest p294 25ul  |  |
|   |  |
|   |  |
|   |  |

Reminders:  
Add Gel Green  
Add Loading Dye  
Run Red

Reagents used:



## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

Date and Time: 8-31-14 @ 7pm Performed by: ANNA GARVEY**Gel Extraction**

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | SOE 1  | SOE 2  | Vector |  |  |  |  |  |  |
|-------------------|--------|--------|--------|--|--|--|--|--|--|
| Tube + Gel weight | 1.077g | 1.087g | 1.101g |  |  |  |  |  |  |
| Empty tube weight | .922g  | .911g  | .916g  |  |  |  |  |  |  |
| Gel Weight        | 0.155  | .176   | .185g  |  |  |  |  |  |  |
| Buffer QG         | .405   | .528   | .555g  |  |  |  |  |  |  |
| Isopropanol       | 0.155  | 0.176  | 0.185  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.

6. Add 1 volume of isopropanol to each tube and mix gently.

7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)

10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: SOE 1 Vector Gel Extract pg. 294Location of Product: Primer Box

Next Step:

Qubit

Date and Time: 8:25pm 8-31-14 Performed by: ANNA GARVEY

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 8-29-14  
Protocol: Ligation  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: ANNA GARVEY  
MATT MORITZEN

Procedure (with applicable notes):

A: 2 $\mu$ L Ligase Buffer, 1 $\mu$ L T4 DNA Ligase, 10 $\mu$ L SOEing, 7 $\mu$ L KB75004

B:

C: -the concentrations of the insert and vector were very low,  
D: thus we did not have enough DNA solution to do proper negative s.

Results:

Location of product: SOE PCR + KB75004 p. 298 AG

Label on product: Primer Box

End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

## Zymo Mini Prep

Name(s): Matthew Merten  
 Date and Time: 8/4/14 9pm

Check off as you  
complete the steps

**Procedure:**

Bacteria used: K875004 (Chlor)

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒  
 Note: This step differs from the printed instructions
2. Resuspend in 525 $\mu$ L of buffer sterile DI..... ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>525\mu$ L
3. Add 100 $\mu$ L 7X Lysis Buffer..... ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Neutralization Buffer..... ☒  
 Inverting 4-6 times immediately
5. Centrifuge 6min at 13000rpm..... ☒
6. Pipet supernatant into a spin column..... ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 200 $\mu$ L Endo-Wash Buffer to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 400 $\mu$ L of Zyppy Wash Buffer to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 30 $\mu$ L Zyppy Elution Buffer to column..... ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: K875004 Chlor Plasmid 9/4/14 mwm p299

Location of product: Plasmid Box

Deviations from Procedure and other Notes: Eluted 2 into the same tube

NEXT STEP: Digested

Continued on back? Yes ☐; No ☐



Date and Time: 9-5-14 6:30pm Performed by: ANNA GARVEY

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: \_\_\_\_\_ Performed by: Matt Mortensen  
Anna Garvey

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- o Buffer NE Buffer 2.1
- o DNA 875004, SOEing PCR Product
- o Enzyme Spe I, Pst I, Xba I

| MATERIALS | TYPE           | AMOUNT USED            |
|-----------|----------------|------------------------|
| Buffer    | NEB            | 2.5 $\mu$ L            |
| DNA       | 875004         | 5 $\mu$ L              |
| Enzyme(s) | Spe I<br>Pst I | 1 $\mu$ L<br>1 $\mu$ L |
| Water     | DEPC           | 15.5 $\mu$ L           |

Total Reaction Volume 25  $\mu$ L

$N_1 = N_0 \text{ Enzyme}$      $N_2 = N_0 \text{ DNA}$

Construct a table with this format for each reaction that you do today.

|         |                |                        |
|---------|----------------|------------------------|
| Buff    | NEB            | 2.5                    |
| DNA     | PCR Prod       | 6 $\mu$ L              |
| Enzymes | Xba I<br>Pst I | 1 $\mu$ L<br>1 $\mu$ L |
| Dep C   | Dep C          | 14.5 $\mu$ L           |

25  $\mu$ L Total

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

**NEXT STEP:**

Date and Time: \_\_\_\_\_ Performed by: Matt Mortensen  
Anna Garvey

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | Vector      | PCR         |  |  |  |  |  |  |  |
|-------------------|-------------|-------------|--|--|--|--|--|--|--|
| Tube + Gel weight | 1.030g      | 1.093       |  |  |  |  |  |  |  |
| Empty tube weight | .925g       | .906g       |  |  |  |  |  |  |  |
| Gel Weight        | .105g       | .187g       |  |  |  |  |  |  |  |
| Buffer QG         | 315 $\mu$ L | 561 $\mu$ L |  |  |  |  |  |  |  |
| Isopropanol       | 105 $\mu$ L | 187 $\mu$ L |  |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100 $\mu$ L per 100mg; so 3 volumes is 300 $\mu$ L per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.
6. Add 1 volume of isopropanol to each tube and mix gently.
7. Load ~800 $\mu$ L of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750 $\mu$ L Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)
10. Load 30 $\mu$ L Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: ?

Location of Product: Primer Box

Next Step:

Date and Time: \_\_\_\_\_ Performed by: Matt Markensén Anna Garvey

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed       
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L       
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200
4. Subtract #3 from #2
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 9/14/14  
Protocol: Ligation  
Reagents: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Technicians(s) involved: Matthew Mortensen  
Anna Garvey

## Procedure (with applicable notes):

- 2x A: 1  $\mu$ L T4 DNA Ligase, 2  $\mu$ L Ligase Buffer, 4.76  $\mu$ L 875004 Gel Extraction (p302),  
6.21  $\mu$ L SOE Gel extract (p302), 6.03  $\mu$ L Dep C Water  
B: 0  $\mu$ L T4 DNA Ligase, 2  $\mu$ L Ligase Buffer, 4.76  $\mu$ L 875004 Gel Extraction (p302)  
6.21  $\mu$ L SOE Gel extract (p302), 7.03  $\mu$ L Dep C  
C: 1  $\mu$ L T4 DNA, 2  $\mu$ L Ligase Buffer, 4.76  $\mu$ L 875004, 0  $\mu$ L SOE, 12.24  $\mu$ L Dep C

## Results:

Location of product: Electroporated  
Label on product: \_\_\_\_\_  
End Notes/Comments: \_\_\_\_\_

## NEXT STEP:

Continued on back? Yes ☐; No ☐



Name(s): ANNA GARVEY  
MATT MORTENSEN

## Electroporation

Page: 305

Reagents: \_\_\_\_\_

### Cell Preparation

Competent cells thawed? Yes ☒ No \_\_\_\_\_ Amount? 40  $\mu$ l

### Electroporation

Electroporation cuvette chilled? Yes ☒ No \_\_\_\_\_

Amount of SOC added to culture tubes: 1 mL LB

DNA placed in m/f tubes? Yes ☒ No \_\_\_\_\_

Placed on ice? Yes \_\_\_\_\_ No \_\_\_\_\_

Amount of DNA mixed with competent cells: 3  $\mu$ l

Voltage used for electroporation: 200

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No \_\_\_\_\_

Amount added: 4 to 5 mL

Cells shaken at 37°C? Yes ☒ No \_\_\_\_\_

Start time: \_\_\_\_\_ End time: \_\_\_\_\_

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes ☒ No \_\_\_\_\_

Cells spun in centrifuge (10-15s)? Yes \_\_\_\_\_ No \_\_\_\_\_

s/n decanted off? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells resuspended? Yes \_\_\_\_\_ No \_\_\_\_\_

Amount of solution: \_\_\_\_\_

Dilutions performed:

$10^{-1}$  Yes ☒ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-2}$  Yes ☒ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-3}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-4}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-5}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-6}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-7}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-8}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-9}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: AX-17  $10^{-n}$  AG 9-14-14

Location of products: Warm

Next steps: Room

Signature: \_\_\_\_\_

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

## Mini Prep

Name(s): Matt Montonen  
Anna Garvey  
 Date and Time: 9-21-14

Check off as you  
complete the steps

**Procedure:**  $\phi$   $\frac{Hac}{Bac}$  + RBS + cuts;  $\phi$  UC57 (Amp)

Bacteria used:  $\phi$  Bac

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
2. Resuspend in 250 $\mu$ L of buffer P1..... ☐  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☐  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☐  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☐  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☐  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 50 $\mu$ L Buffer EB to column..... ☐  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: \_\_\_\_\_

Location of product: \_\_\_\_\_

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: \_\_\_\_\_ Performed by: \_\_\_\_\_

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed \_\_\_\_
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_
4. Subtract #3 from #2 \_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 4:30 pm Performed by: Matt Murken**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- Buffer \_\_\_\_\_
- DNA \_\_\_\_\_
- Enzyme \_\_\_\_\_

| MATERIALS | TYPE                         | AMOUNT USED             |
|-----------|------------------------------|-------------------------|
| Buffer    | NEB 2.1                      | 2.5 $\mu$ L             |
| DNA       | pBAD-RBS- <i>cat</i> #2 p306 | 3.7 $\mu$ L (1 $\mu$ g) |
| Enzyme(s) | <i>Nhe</i> I<br><i>Pst</i> I | 1 $\mu$ L<br>1 $\mu$ L  |
| Water     | DEPC                         | 16.8                    |

Total Reaction Volume 25  $\mu$ L

Construct a table with this format for each reaction that you do today.

NEB 2.1 2.5  $\mu$ LK215104 Miniprep B 4.5  $\mu$ L (1  $\mu$ g)  
p212*Xba*I 1  $\mu$ L  
*Pst*I 1  $\mu$ LDepc 16  $\mu$ L25  $\mu$ L total

Start Time of Incubation: 5:20

End Time of Incubation: 6:20

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°

Method used for Quenching the Reaction (*different for different enzymes*)

80°C Heat shock for 20°C

Where did you store your finished product and what did you label it?

\_\_\_\_\_

NEXT STEP:

Date and Time: \_\_\_\_\_  
 Protocol: Ligation  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

L<sub>1</sub>      L<sub>2</sub>      L<sub>3</sub>      L<sub>4</sub>  
 Procedure (with applicable notes):

|  |              |               |           |              |
|--|--------------|---------------|-----------|--------------|
| Ligase Buffer  | 2 $\mu$ L    | 2 $\mu$ L     | 2 $\mu$ L | 2 $\mu$ L    |
| T4 DNA Ligase  | 1 $\mu$ L    | 1 $\mu$ L     | 0 $\mu$ L | 1 $\mu$ L    |
| Vector pBAD-RBS+<br>cut site<br>p308<br>insert<br>K245004<br>p225<br>Dep C | 1.25 $\mu$ L | 1.25          | 1.25      | 1.25 $\mu$ L |
|  | 5.94 $\mu$ L | 11.88 $\mu$ L | 5.94      | 5.94         |
|  | 9.81 $\mu$ L | 3.87          | 10.81     | 11.06        |

Results:

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: Transformed p310

NEXT STEP:

Continued on back? Yes ☐; No ☐

Name(s): ANNA GARVEY  
MATT MORRISON

## Electroporation

Page: 310

Reagents: \_\_\_\_\_

### Cell Preparation

Competent cells thawed? Yes X No \_\_\_\_\_ Amount? 405 mL

### Electroporation

Electroporation cuvette chilled? Yes X No \_\_\_\_\_

Amount of SOC added to culture tubes: 1 mL

DNA placed in m/f tubes? Yes X No \_\_\_\_\_

Placed on ice? Yes \_\_\_\_\_ No \_\_\_\_\_

Amount of DNA mixed with competent cells: 2  $\mu$ L

Voltage used for electroporation: ECOL

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes X No \_\_\_\_\_

Amount added: 5 mL

Cells shaken at 37°C? Yes X No \_\_\_\_\_

Start time: \_\_\_\_\_ End time: \_\_\_\_\_

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes \_\_\_\_\_ No X

Cells spun in centrifuge (10-15s)? Yes \_\_\_\_\_ No X

s/n decanted off? Yes \_\_\_\_\_ No X

Cells resuspended? Yes \_\_\_\_\_ No X

Amount of solution: \_\_\_\_\_

Dilutions performed:

$10^{-1}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-2}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-3}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-4}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-5}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-6}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-7}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-8}$  Yes \_\_\_\_\_ No \_\_\_\_\_

$10^{-9}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: PBAD + tag + pump on PUC57 59-23-147

Location of products: Warm Room

Next steps: \_\_\_\_\_

Signature: \_\_\_\_\_

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>



Date and Time: \_\_\_\_\_ Performed by: \_\_\_\_\_

**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEBuffer 2.1
- o DNA K314200 psBIC3 miniprep product 70.6 ng/ul (p273,274)
- o Enzyme XbaI PstI

| MATERIALS | TYPE                  | AMOUNT USED |
|-----------|-----------------------|-------------|
| Buffer    | NEB 2.1               | 5ul         |
| DNA       | K314200 psBIC3 (p273) | 28.3ul      |
| Enzyme(s) | XbaI<br>PstI          | 1ul<br>1ul  |
| Water     | DEPC                  | 14.7ul      |

Total Reaction Volume 50ul

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: 8:30

End Time of Incubation: 9:30

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): 37°C

Method used for Quenching the Reaction (*different for different enzymes*)

20 min 80°C Heat Quenching

Where did you store your finished product and what did you label it?

NEXT STEP:

## Mini Prep

Name(s): ANNA GARVEY  
 Date and Time: 9-25-14 3:10pm

Check off as you  
complete the steps

**Procedure:**

Bacteria used: L<sub>2</sub> -> pBAD + RBS + pump <sup>Strong</sup>

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1..... ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: <colony #> pBAD + RBS + pump  
 Location of product: primer box

Deviations from Procedure and other Notes: Only cultures 4, 6, 9, + 10 grew

Successful?

NEXT STEP:

gcl

Continued on back? Yes ☐; No ☐

Date and Time: \_\_\_\_\_ Performed by: \_\_\_\_\_

**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer \_\_\_\_\_
- o DNA \_\_\_\_\_
- o Enzyme \_\_\_\_\_

| MATERIALS | TYPE   | AMOUNT USED                  |
|-----------|--|------------------------------|
| Buffer    | NEB Buffer 2.1   | 2.5 $\mu$ L                  |
| DNA       | pBAD+PBS+Ca <sub>4</sub> St <sub>4</sub> te<br>P306 (272 $\mu$ S/mL) | 1.84 $\mu$ g<br>(.5 $\mu$ g) |
| Enzyme(s) | <del>Not</del> NheI<br>PstI  | .5 $\mu$ L<br>.5 $\mu$ L     |
| Water     | DEPC   | 19.66 $\mu$ L                |

Total Reaction Volume 25

21.16 For - Enzyme Negatives

Construct a table with this format for each reaction that you do today.

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

NEXT STEP:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: \_\_\_\_\_

Lane Reagents

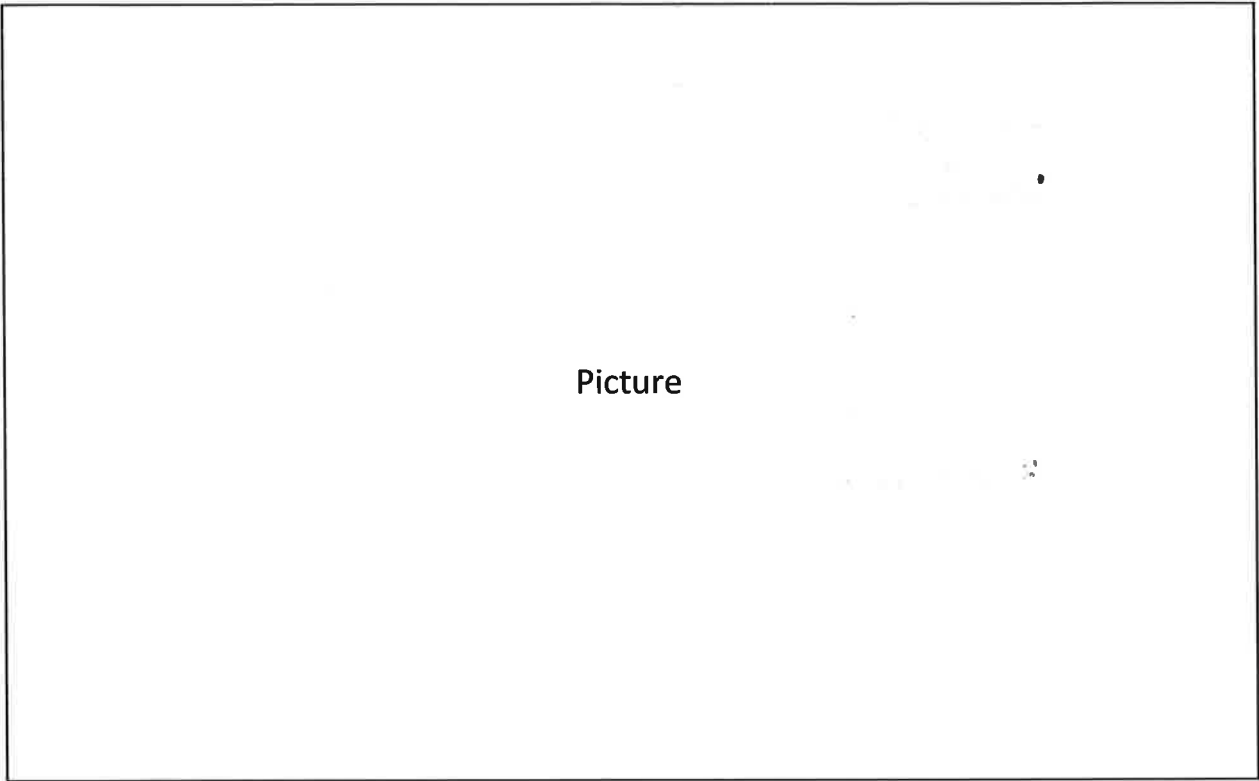
|   |    |                   |   |
|---|----|-------------------|---|
| 1 | L  | Ladder 1kb        | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | 4  | Transformation #4 |   |
| 3 | 6  | #6                |   |
| 4 | 9  | #9                |   |
| 5 | 10 | #10               |   |
| 6 | C  | K314200 Digest    |   |
| 7 |    |                   |   |
| 8 |    |                   |   |

Reagents used:

~~2ul loading dye~~  
~~D3 Digest~~  
~~+P } neg w/ pst 2~~  
~~-N }~~  
~~+N }~~  
~~-P }~~  
~~308 } digested~~  
~~6/6~~

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

NEXT STEP:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: \_\_\_\_\_

Lane Reagents

1

2

3

4

5

6

7

8

Ladder 1kb NEB

~~Miniprep~~ Miniprep Product #9 p312pBAD + RBS + Cut sites <sup>pUC57</sup> from Glycerol stock - Miniprep

pBAD + RBS + Cut sites pUC57 from Glycerol stock

K25104 "pump" Gel Extract p325

Reminders:

Add Gel Green

Add Loading Dye

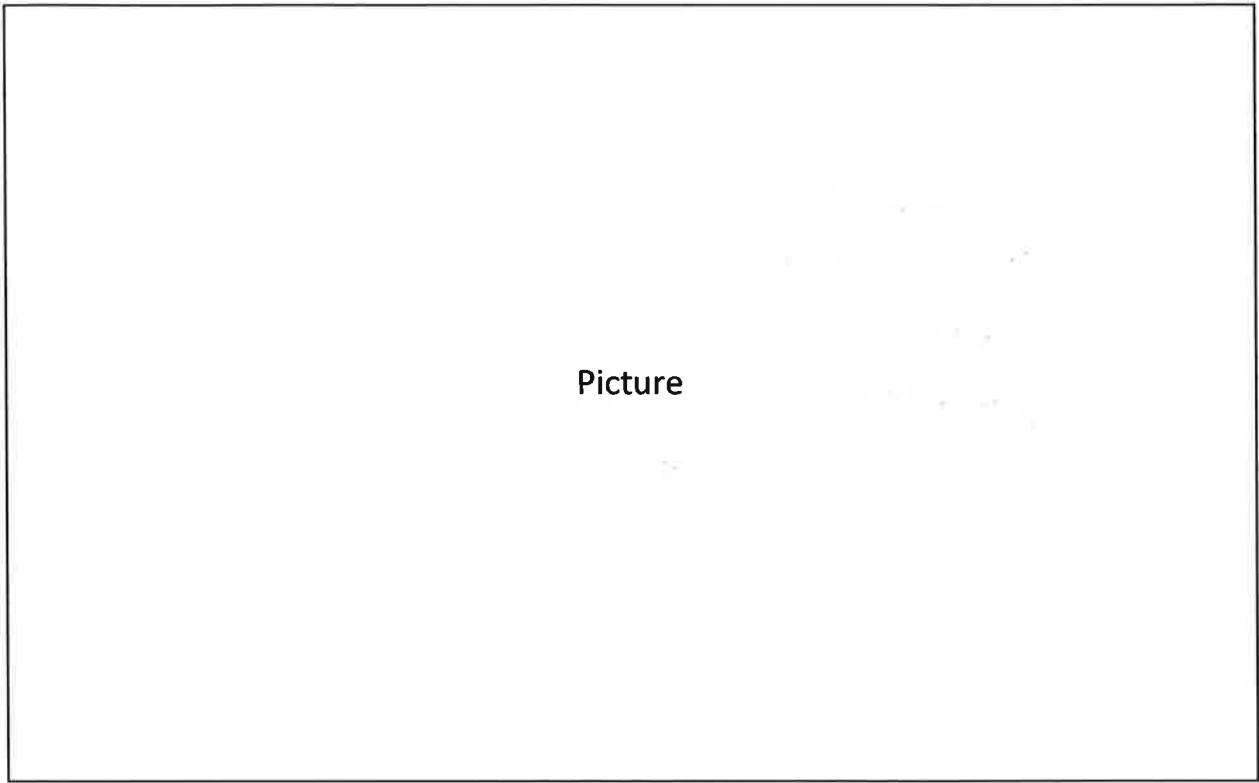
Run Red

Reagents used:



Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Results

NEXT STEP:

# Gel Electrophoresis

Page 316

Name: Serra Tackett

Date: \_\_\_\_\_

Time: 12:10

% Agarose: 1

Lane Reagents

|   |     |                                     |   |
|---|-----|-------------------------------------|---|
| 1 | 2 L | Ladder 25uL + 1uL loading dye       | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | 3 D | 10uL <del>QIA</del> 2uL loading dye |   |
| 3 | 4 P |                                     |   |
| 4 | 5 N |                                     |   |
| 5 | 6 Z |                                     |   |
| 6 |     |                                     |   |
| 7 |     |                                     |   |
| 8 |     |                                     |   |

Reagents used:

D - Digest

P +P } neg w/ Pst I  
-N }

N +N }  
-P }

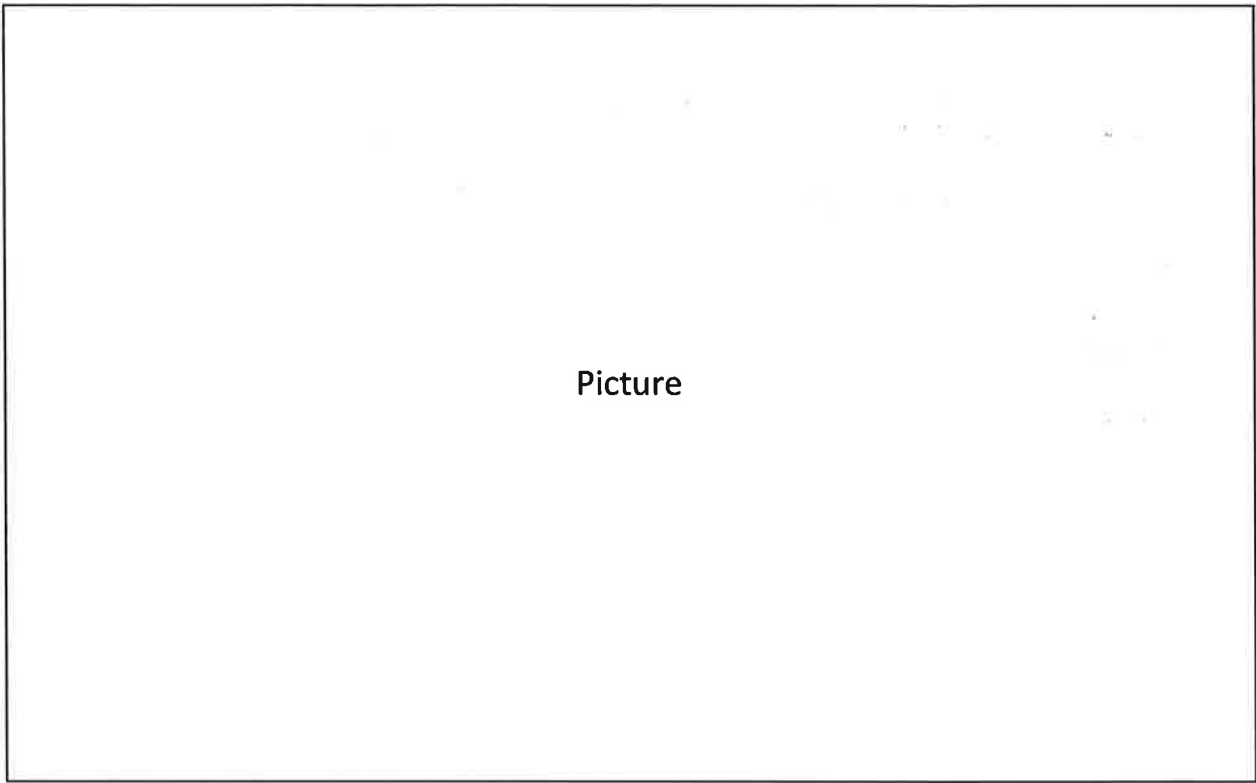
Z 308 } pBad + RBS + outside pu57 CAMP1  
Digest 9/23/14

Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_



Results

NEXT STEP:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: \_\_\_\_\_

Lane Reagents

1

2

3

4

5

6

7

8

L

P

+N  
-P+P  
-N

Dig

Old Dig

Reminders:

Add Gel Green

Add Loading Dye

Run Red

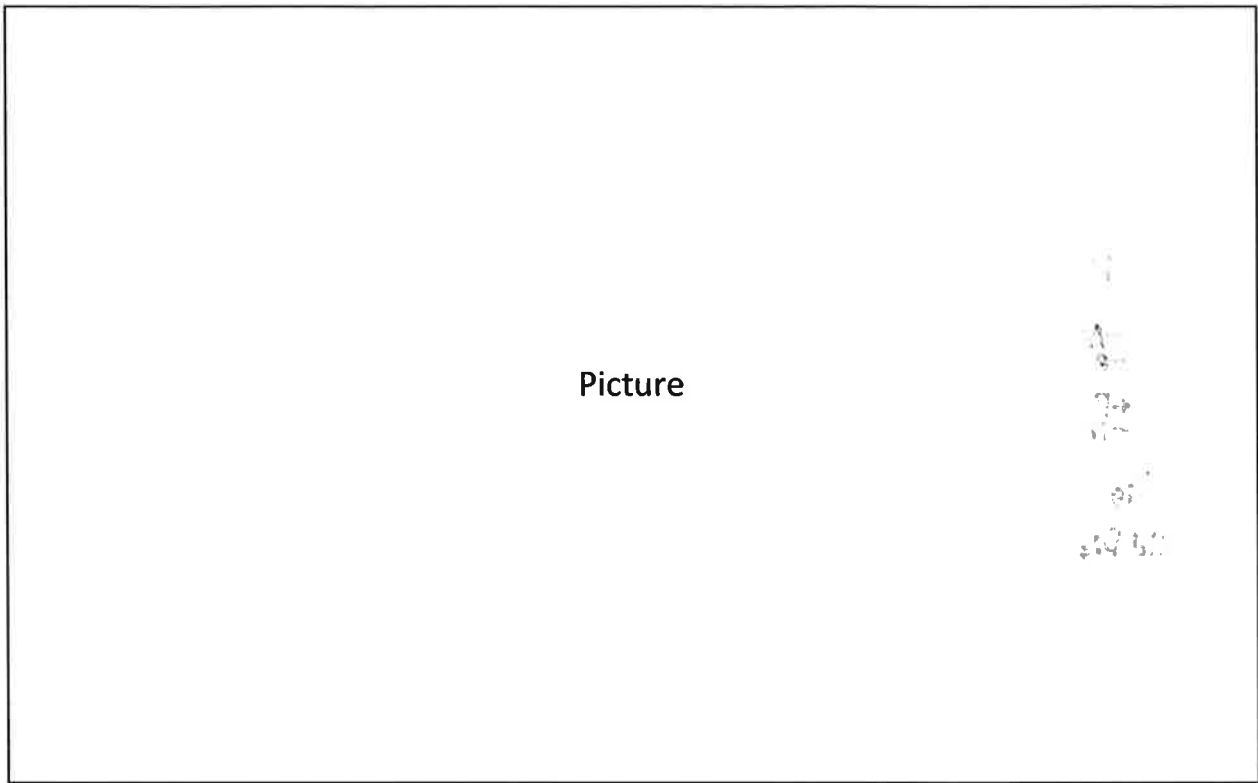
Reagents used:

Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_



Results

NEXT STEP:

## Mini Prep

Name(s): \_\_\_\_\_

Date and Time: \_\_\_\_\_

Check off as you  
complete the steps**Procedure:**

Bacteria used: \_\_\_\_\_

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
2. Resuspend in 250 $\mu$ L of buffer P1..... ☐  
Transfer resuspended bacteria to microcentrifuge tube- the total will be >250 $\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☐  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☐  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☐  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☐  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 50 $\mu$ L Buffer EB to column..... ☐  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in -20°C for later use..... ☐

Label on centrifuge tube: \_\_\_\_\_

Location of product: \_\_\_\_\_

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: \_\_\_\_\_ Performed by: Matt Markson  
Anna Garvey

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed \_\_\_\_\_
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_\_
4. Subtract #3 from #2 \_\_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]



Date and Time: 9-29-14 9:30 pm Performed by: Matthew Martensen  
Anna Garvey

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- Buffer \_\_\_\_\_
- DNA \_\_\_\_\_
- Enzyme \_\_\_\_\_

| MATERIALS | TYPE | AMOUNT USED |
|-----------|------|-------------|
| Buffer    |      |             |
| DNA       |      |             |
| Enzyme(s) |      |             |
| Water     | DEPC |             |

Total Reaction Volume \_\_\_\_\_

Construct a table with this format for each reaction that you do today.

|         | Type                         | Amount                   |                          |
|---------|------------------------------|--------------------------|--------------------------|
| Buffer  | 2.1                          | 2.5 uL                   | Negative 2.5 uL          |
| DNA     | K114200<br>pSB1C3 #1<br>p319 | 20.5 (negative negative) | 12.2 uL                  |
| Enzymes | Xba I<br>Pst I               | 1 uL<br>1 uL             | .5 uL or 0<br>.5 uL or 0 |
| Water   | —                            | —                        | 9.8 uL                   |

Negative

|            |      | Negative |
|------------|------|----------|
| NEB 3.1    | 2.5  | 2.5      |
| pBAD+RBS 2 | 10.2 | 5.1      |
| Cut sites  | 1 uL | .5 or 0  |
| Sma I      | 1 uL | .5 or 0  |
| Bam HI     |      |          |
| Water      | 10.3 | 16.9     |
| DEPC       |      |          |

-5 = -X  
 -4 = -P

|            |      | Negative   |
|------------|------|------------|
| NEB 3.1    | 2.5  | 2.5        |
| pLac+RBS 2 | 20.5 | 8.36       |
| Cut sites  |      |            |
| Sma I      | 1 uL | .5 uL or 0 |
| Bam HI     | 1 uL | .5 uL or 0 |
| DEPC       | 0    | 13.64      |

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

**NEXT STEP:**

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: \_\_\_\_\_

Lane Reagents

1

2

3

4

5

6

7

8

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Ladder

314200 (SB/C) +X+P

+X-P

-X+P

p320

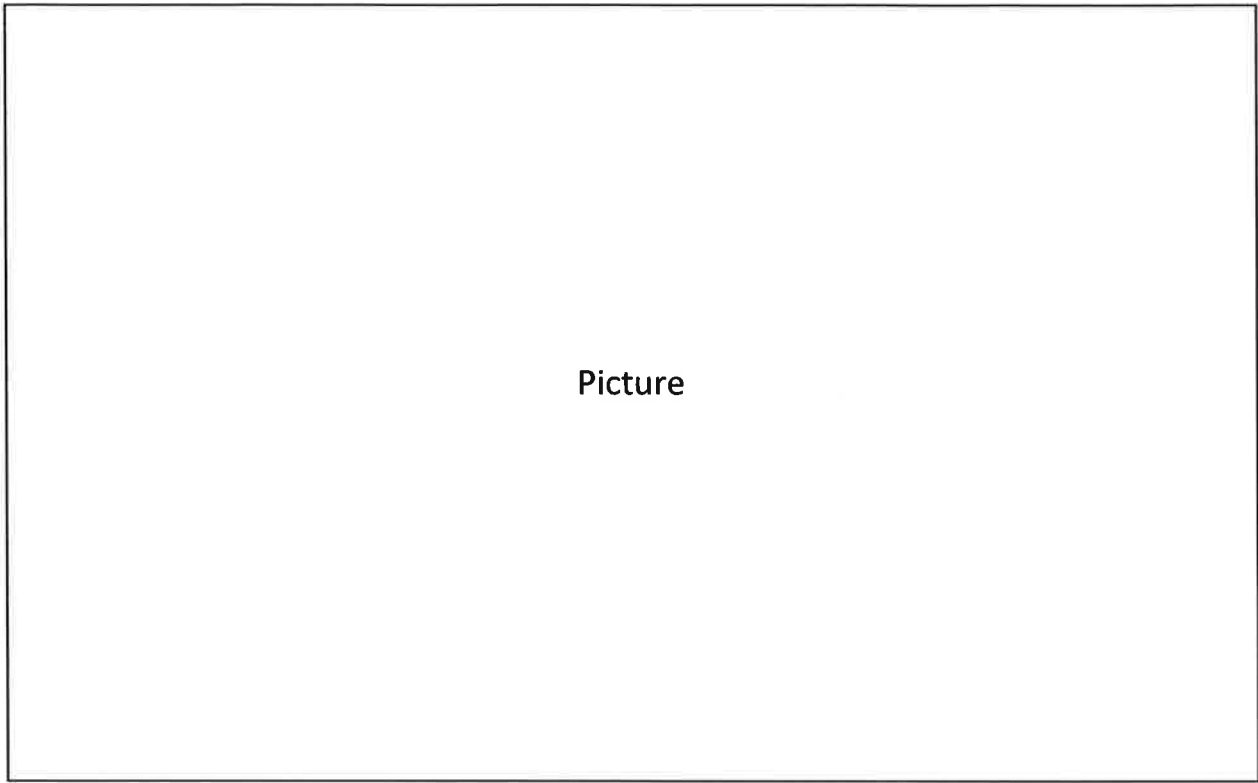
Reagents used:

Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_



Results

|            |
|------------|
| NEXT STEP: |
|------------|

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: \_\_\_\_\_

Lane Reagents

|   |                           |             |        |   |
|---|---------------------------|-------------|--------|---|
| 1 | pBAD                      | -X+P        | } p320 | Reminders:<br>Add Gel Green<br>Add Loading Dye<br>Run Red |
| 2 | pBAD                      | +X-P        |        |   |
| 3 | pBAD <sup>RBS</sup> + cut | +X+P Digest |        |   |
| 4 | Ladder                    |             |        |   |
| 5 | pLac                      | +X+P Digest |        |   |
| 6 | pLac                      | +X-P        |        |   |
| 7 | pLac                      | -X+P        |        |   |
| 8 |                           |             |        |   |

Reagents used:

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

## Mini Prep

Name(s): Matt Murtaw  
 Date and Time: 8:40 am 9/30/14

Check off as you  
complete the steps

**Procedure:**

Bacteria used: K314200 258/C3

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant. ☐
2. Resuspend in 250 $\mu$ L of buffer P1. ☐  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2. ☐  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3. ☐  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm. ☐
6. Pipet supernatant into a QIAprep spin column. ☐  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column. ☐  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column. ☐  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube. ☐
10. Add 50 $\mu$ L Buffer EB to column. ☐  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through. ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use. ☐

Label on centrifuge tube: \_\_\_\_\_

Location of product: \_\_\_\_\_

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: \_\_\_\_\_  
Protocol: Gel Extract  
Reagents: 9:30 pm 9/20/14

Lab Technicians(s) involved: Matt Markensen

PSB/C3  
Procedure (with applicable notes):

|            |                          |                          |                           |
|------------|--------------------------|--------------------------|---------------------------|
| Empty Tube | <del>0.00</del><br>.911g | <del>0.00</del><br>.916g | <del>0.00</del><br>.9127g |
| Tube + Gel | 1.007g                   | 1.106g                   |                           |
| Gel        | .096g                    | .190g                    |                           |
| QG         | 288uL                    | 570uL                    |                           |

Results:

Location of product: \_\_\_\_\_  
Label on product: \_\_\_\_\_  
End Notes/Comments: Both cut w/ X+P

NEXT STEP:

Continued on back? Yes ☐; No ☐



## Mini Prep

Name(s): M. H. Munksgaard

Date and Time: \_\_\_\_\_

Check off as you  
complete the steps**Procedure:**Bacteria used: plasmid 3 pUC57 pUC57  
K21544 P8AD+R844 PL4 + R844

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☐
2. Resuspend in 250 $\mu$ L of buffer P1..... ☐  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☐  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☐  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☐
6. Pipet supernatant into a QIAprep spin column..... ☐  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☐  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☐  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☐
10. Add 50 $\mu$ L Buffer EB to column..... ☐  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☐
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☐

Label on centrifuge tube: \_\_\_\_\_

Location of product: \_\_\_\_\_

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: \_\_\_\_\_ Performed by: Matthew Mortensen

## Qubit DNA Quantification

### Master Mix:

1. Find out how many samples will need to be analyzed 3
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L \_\_\_\_\_
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 \_\_\_\_\_
4. Subtract #3 from #2 \_\_\_\_\_
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### Standard and Sample Preparation:

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### Running Samples

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

21.48

[illegible]

## Mini Prep

Name(s): ANNA GARVEY  
Date and Time: 10-2-14 7:30

Check off as you  
complete the steps

**Procedure:**

Bacteria used: PLAC + RBS + cut / pBAD + RBS + cut / K215104

1. Pellet bacteria via centrifuge at  $\geq 8000$  rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1..... ☒  
Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
Centrifuge 30-60sec then discard flow through  
Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: Orange "Pump" Box  
Location of product: (pBAD) + RBS + (cut site) (or 214105) 2 10-2-14

Deviations from Procedure and other Notes:

Successful?

NEXT STEP:

Qubit La Perla  
(Pearl?) Ask Drs. Herkel or Werner  
to show how to use.

Continued on back? Yes ☐; No ☐

Date and Time: 10-3-14 6:00pm Performed by: Matthew Mortensen + Anna Garvey

## **Qubit DNA Quantification Ver 1.0**

### **Master Mix:**

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

Date and Time: 10-3-14 6:30pm Performed by: ANNA GARVEY + MATTHEW MOTTENSEN

## Restriction Enzyme Digest

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

## PROCEDURE

### Material Location

- Buffer NEB 2.1
- DNA CPBAD + SPAC + RBS + cutsite (K215104 + RBS + cutsite)
- Enzyme XbaI, PstI

| MATERIALS | TYPE | AMOUNT USED |
|-----------|------|-------------|
| Buffer    |      |             |
| DNA       |      |             |
| Enzyme(s) |      |             |
| Water     | DEPC |             |

Total Reaction Volume 25  $\mu$ L

Construct a table with this format for each reaction that you do today.

|                      |   |  |
|----------------------|---|--|
| PLAC + RBS + cutsite |   |  |
| Buffer NEB 2.1       | 2.5 $\mu$ L   | $N_1 = -XbaI + RBS + I$                      |
| Enzymes XbaI PstI    | <del>2.5 <math>\mu</math>L</del><br>2 $\mu$ L       | $N_2 = +XbaI - PstI$                         |
| DNA                  | <del>13.34 <math>\mu</math>L</del><br>13.34 $\mu$ L |  |
| Dep C                | <del>10.03 <math>\mu</math>L</del><br>10.03 $\mu$ L |  |
|                      |   | $N_1 = +XbaI + RBS + I$ $N_2 = +XbaI - PstI$ |

|                      |   |  |
|----------------------|---|--|
| PBAD + RBS + cutsite |   |  |
| Buffer (2.1)         | 2.5 $\mu$ L   |  |
| Enzymes XbaI PstI    | <del>2.5 <math>\mu</math>L</del><br>2 $\mu$ L       |  |
| DNA                  | <del>7.87 <math>\mu</math>L</del><br>7.87 $\mu$ L   |  |
| Dep C                | <del>10.03 <math>\mu</math>L</del><br>10.03 $\mu$ L |  |
|                      |   | $N_1 = +XbaI + RBS + I$ $N_2 = +XbaI - PstI$ |

|                   |   |  |
|-------------------|---|--|
| K215104 (PSB1C3)  |   |  |
| Buffer (2.1)      | 2.5 $\mu$ L   |  |
| Enzymes XbaI PstI | <del>2.5 <math>\mu</math>L</del><br>2 $\mu$ L       |  |
| DNA               | <del>14.18 <math>\mu</math>L</del><br>14.18 $\mu$ L |  |
| Dep C             | <del>4.32 <math>\mu</math>L</del><br>4.32 $\mu$ L   |  |
|                   |   | $N_1 = -XbaI + RBS + I$ $N_2 = +XbaI - PstI$ |

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

**NEXT STEP:**



Date and Time: 10-4-14 5:30 Performed by: Anna Garvey

## **Qubit DNA Quantification**

### **Master Mix:**

1. Find out how many samples will need to be analyzed ✓
  - Note: You will need enough master mix for two more (The standards)
2. Take #1 and add two samples, then multiply this by 200 $\mu$ L and add an extra 100 $\mu$ L ✓
  - Note: the extra 100 $\mu$ L is added because you lose a bit of mix every time you pipette; this is because pipettes are calibrated to deliver exactly what they have dialed, but they pick up extra reagent.
3. Take #2 and divide it by 200 ✓
4. Subtract #3 from #2 ✓
5. Find a tube capable of holding #2 worth of liquid
  - Note: a micro-centrifuge or a conical tube will do; sterility is not extremely important for this, as the reagents will be thrown away once the experiment is done.
6. Pipette #4 of Component B Quant-iT dsDNA BR Buffer (in the kit) into your collection tube.
7. Add #3 of 200X Quant-iT dsDNA BR Reagent
8. Vortex lightly to mix.

### **Standard and Sample Preparation:**

1. In a Qubit assay tube mix 190 $\mu$ L Master Mix with 10 $\mu$ L Quant-iT BR Standard #1. Mix by tapping the tube on the table 5-10 times.
2. Repeat Step #1 for Quant-iT BR Standard #2
  - Note: Qubit assay tubes are bigger than PCR tubes and smaller than Micro-centrifuge tubes; Only Qubit tubes will work.
  - Note: The standards should be in the 4°C
  - Note: Only write on the caps of Qubit tubes when labeling them
3. For each of your samples in individual Qubit tubes, mix 199 $\mu$ L master mix with 1 $\mu$ L DNA

### **Running Samples**

Note: standards and samples should sit for at least 2 minutes before being run. They remain viable for up to 3 hours.

1. At the Qubit's home screen, select "Run New Calibration", then select "Quant-iT dsDNA BR"
2. Insert Standard 1 and hit Go. When prompted, enter Standard 2 and press Go.
3. If calibration is successful, insert a sample and press Go. After a few seconds, you will be given the concentration of nucleic acid in the tube, **don't use this number**. Select "Calculate Sample Concentration" and then select 1 $\mu$ L; record the number you get.
4. Repeat step #3 for each sample
5. Once all of your samples have been run, simply discard the tubes.

## Results

[illegible]

## Mini Prep

Name(s): ANNA GARVEY  
 Date and Time: 10-4-14

Check off as you  
complete the steps

**Procedure:**

Bacteria used: PLAC + RBS + cut / pBAD + RBS + cut site / K1210-1 / pET58

1. Pellet bacteria via centrifuge at  $\geq 8000$ rpm for 3min. Discard supernatant..... ☒
2. Resuspend in 250 $\mu$ L of buffer P1..... ☒  
 Transfer resuspended bacteria to microcentrifuge tube- the total will be  $>250\mu$ L
3. Add 250 $\mu$ L Buffer P2..... ☒  
 Invert 4-6 times (less than 5 minutes until next step)
4. Add 350 $\mu$ L Buffer N3..... ☒  
 Inverting 4-6 times immediately
5. Centrifuge 10min at 13000rpm..... ☒
6. Pipet supernatant into a QIAprep spin column..... ☒  
 Centrifuge 30-60sec then discard flow through  
 Note: If multiple cultures of the same type are used, they are combined in this step
7. Add 500 $\mu$ L Buffer PB to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
8. Add 750 $\mu$ L of Buffer PE to column..... ☒  
 Centrifuge 30-60sec and then discard flow through
9. Transfer column to new microcentrifuge tube..... ☒
10. Add 50 $\mu$ L Buffer EB to column..... ☒  
 Let stand for 60sec then centrifuge for 60sec
11. Discard column and save centrifuge tube with flow through..... ☒
12. Label and store in  $-20^{\circ}\text{C}$  for later use..... ☒

Label on centrifuge tube: K1210-1 (unbo) 10-4-14 AG

Location of product: Orange Box

Deviations from Procedure and other Notes:

Successful?

Yes (see Quantification pg. 331)

NEXT STEP:

Continued on back? Yes ☐; No ☐

Date and Time: \_\_\_\_\_  
 Protocol: PCR \_\_\_\_\_  
 Reagents: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Lab Technicians(s) involved: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Procedure (with applicable notes):

|               |       |      |       |      |       |      |
|---------------|-------|------|-------|------|-------|------|
| Master Mix:   | Xyn A | -    | Bg15  | -    | Yes2  | -    |
| Primer 1      | 12.5  | 12.5 | 12.5  | 12.5 | 12.5  | 12.5 |
| Primer 2      | 2uL   | -    | 2uL   | =    | 2uL   | -    |
| Genomic DNA   | 8.5uL | 4.5  | 8.5uL | 4.5  | 8.5uL | 4.5  |
| (Bsu sub 168) | 0     |      |       |      |       |      |
| Dep C         | 0uL   | 8uL  | 0uL   | 8uL  | 0uL   | 8uL  |

45° Denaturation  
 63° Annealing x 34 cycles  
 72° Extension

Results:

Location of product: \_\_\_\_\_  
 Label on product: \_\_\_\_\_  
 End Notes/Comments: \_\_\_\_\_

NEXT STEP:

Continued on back? Yes ☐; No ☐

## Gel Electrophoresis

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

% Agarose: \_\_\_\_\_

Lane Reagents

1

2

3

4

5

6

7

8

L. Jeter

X<sub>yn</sub>A PCRX<sub>yn</sub>A Negative

BgIs PCR

BgIs Negative

YesZ PCR

YesZ Negative

p 332  
17uL

Reminders:

Add Gel Green

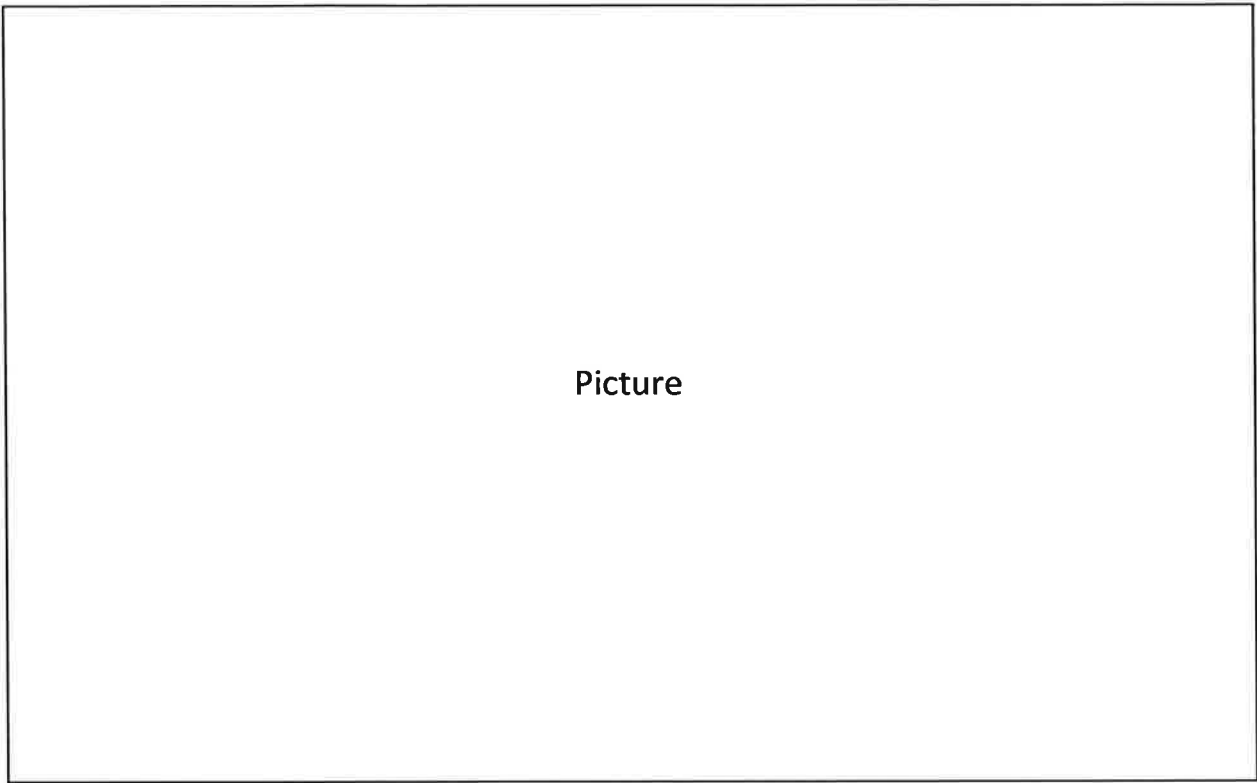
Add Loading Dye

Run Red

Reagents used:

Gel Electrophoresis

Name: \_\_\_\_\_  
Date : \_\_\_\_\_  
Time: \_\_\_\_\_



Picture

Results

|            |
|------------|
| NEXT STEP: |
|------------|

Date and Time: 10-5-14 10:05 Performed by: ANNA GARVEY**Restriction Enzyme Digest**

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

**PROCEDURE****Material Location**

- o Buffer NEB 2.1
- o DNA <P<sup>A</sup>BAD> <P<sup>A</sup>LAC> <K215104> <P<sup>B</sup>ET286> pg. 331
- o Enzyme XbaI, PstI SaI, BamHI

**Double Reaction**

| MATERIALS | TYPE             | AMOUNT USED  |
|-----------|------------------|--------------|
| Buffer    | 2.1              | 25.0 uL      |
| DNA       | PBAD+RBS+cutsite | 7.41 uL      |
| Enzyme(s) | XbaI<br>PstI     | 2 uL<br>2 uL |
| Water     | DEPC             | 8.59 uL      |

Negatives are 1/2 reactions

Total Reaction Volume 25 uL

$N_1 = +XbaI \quad N_2 = -XbaI$   
 $-PstI \quad +PstI$   
 2.5 Buffer  
 1.85 DNA  
 1 Enzyme 1 uL  
 Water 19.65 uL

Double Reaction  
 PLAC + RBS + cutsite

|         |                       |              |
|---------|-----------------------|--------------|
| Buffer  | 2.1                   | 5.0 uL       |
| DNA     | PLAC + RBS + cutsites | 12.4 uL      |
| Enzymes | XbaI<br>PstI          | 2 uL<br>2 uL |
| Water   | DEPC                  | 3.1 uL       |

$N_1 = +XbaI \quad N_2 = -XbaI$   
 $-PstI \quad +PstI$

Buffer 2.5 uL  
 DNA 3.1 uL  
 Enzymes 1 uL  
 water 18.4 uL

Construct a table with this format for each reaction that you do today.

K215104 - Double reaction

|         |                       |              |
|---------|-----------------------|--------------|
| Buffer  | 2.1                   | 5.0 uL       |
| DNA     | PLAC + RBS + cutsites | 12.58 uL     |
| Enzymes | XbaI<br>PstI          | 2 uL<br>2 uL |
| Water   | DEPC                  | 3.42 uL      |

$N_1 = +XbaI \quad N_2 = -XbaI$   
 $-PstI \quad +PstI$

Buffer 2.5 uL  
 DNA 3.145 uL  
 Enzymes 1 uL  
 Water 18.35 uL

PET28a<sup>(water)</sup>

|         |              |              |
|---------|--------------|--------------|
| Buffer  | 2.1          | 5.0 uL       |
| DNA     | PET28a       | 20.5 uL      |
| Enzymes | BamI<br>SalI | 1 uL<br>1 uL |
| Water   | DEPC         | —            |

~~K215104 - Double reaction~~

Negatives from Tube A  
 Buffer 2.5  
 DNA 12.5  
 Enzyme 1 uL  
 Water 9 uL

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

**NEXT STEP:**



## Gel Electrophoresis

Name: Anna Garey + MattDate: 10-6-14Time: 2:45pm% Agarose: 0.8%

Lane Reagents

1

2

3

4

5

6

7

8

K215104N2

K215104N1

K215104

Ladder

pLAC N2

~~pLAC N2~~

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

## Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

NEXT STEP:

## Gel Electrophoresis

Name: ANNA GARVEYDate: 10-6-14Time: 12:45

% Agarose: \_\_\_\_\_

Lane Reagents

1

2

3

4

5

6

7

8

| PLAC N<sub>1</sub>

| PLAC + RBS + cut site

| Ladder

| PBAD + RBS + cut site

| PBAD N<sub>1</sub>| PBAD N<sub>2</sub>

Reminders:

Add Gel Green

Add Loading Dye

Run Red

Reagents used:

# Gel Electrophoresis

Name: \_\_\_\_\_

Date : \_\_\_\_\_

Time: \_\_\_\_\_

Picture

Results

Successful:

NEXT STEP:

Date and Time: 10-16-14 11:45pm Performed by: ANNA GARVEY

## Gel Extraction

Note: Before doing this, a Qubit must be run to determine DNA concentrations

1. Weigh enough sterile micro centrifuge tubes for all of your samples and record.
2. Excise the desire DNA from the gel, being careful to get the entire band, but minimizing excess gel.
3. Weigh the tubes with the DNA samples inserted. Calculate gel weight.

| Sample            | pLAC   | pBAD   | V215104 |  |  |  |  |  |  |
|-------------------|--------|--------|---------|--|--|--|--|--|--|
| Tube + Gel weight | ✓      | ✓      | ✓       |  |  |  |  |  |  |
| Empty tube weight | 0.941g | 0.941g | 0.941g  |  |  |  |  |  |  |
| Gel Weight        | 0.153g | 0.241g | 0.158g  |  |  |  |  |  |  |
| Buffer QG         | 459μL  | 723μL  | 474μL   |  |  |  |  |  |  |
| Isopropanol       | 158μL  | 241μL  | 158μL   |  |  |  |  |  |  |

4. Add 3 Volumes of Buffer QG (per volume of Gel) to each tube

Note: For this protocol, 1 volume is 100μL per 100mg; so 3 volumes is 300μL per 100mg

5. Put the Samples in a heat block at 50°C for 10 minutes, vortexing every 2-3 minutes.

6. Add 1 volume of isopropanol to each tube and mix gently.

7. Load ~800μL of the mixture into the spin column, spin for 1 minute and discard the flow-through.

Note: Repeat step 7 until all of the original mixture has been run through the column.

8. Load 750μL Buffer PE and centrifuge for 1 minute. Discard flow through.

Note: if DNA will be used for sequencing, let the column stand for 2-5 minutes before centrifuging.

9. Place the column (inner portion) into a new, appropriately labeled micro-centrifuge (sterile!)

10. Load 30μL Buffer EB (elution buffer). Let the column stand for at least 1 minute, and spin for 1 minute.

Note: this is the option to try to increase concentration

11. Store

Label on Product: (pBAD + EBS7 cut) Digest Gel Extraction (Amp) 10-16-14 AG

Location of Product: Orange Box

Next Step: Ligate

Name(s): ANNA GARVEY  
MAT MORITENSEN

## Electroporation

Page: 33B

Reagents: \_\_\_\_\_

### Cell Preparation

Competent cells thawed? Yes ☒ No \_\_\_\_\_ Amount? \_\_\_\_\_

### Electroporation

Electroporation cuvette chilled? Yes ☒ No \_\_\_\_\_

Amount of SOC added to culture tubes: 1 mL

DNA placed in m/f tubes? Yes ☒ No \_\_\_\_\_

Placed on ice? Yes ☒ No \_\_\_\_\_

Amount of DNA mixed with competent cells: 2.5  $\mu$ L

Voltage used for electroporation: 1000 V

\*Optimum voltage for *E. coli* is 12,000 to 19,000 V/cm. Using a 0.1cm cuvette means a desired voltage of 1,200 to 1,900 volts.

SOC immediately added after electroporation? Yes ☒ No \_\_\_\_\_

Amount added: 1 mL to <sup>warm</sup> 5 mL

Cells shaken at 37°C? Yes ☒ No \_\_\_\_\_

Start time: \_\_\_\_\_ End time: \_\_\_\_\_

### Serial Dilution

Cells transferred to 1 mL m/f tube? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells spun in centrifuge (10-15s)? Yes \_\_\_\_\_ No \_\_\_\_\_

s/n decanted off? Yes \_\_\_\_\_ No \_\_\_\_\_

Cells resuspended? Yes \_\_\_\_\_ No \_\_\_\_\_

Amount of solution: \_\_\_\_\_

Dilutions performed:

$10^{-1}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-2}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-3}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-4}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-5}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-6}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-7}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-8}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

$10^{-9}$  Yes \_\_\_\_\_ No \_\_\_\_\_

Volumes: \_\_\_\_\_

Labels on products: \_\_\_\_\_

Location of products: \_\_\_\_\_

Next steps: \_\_\_\_\_

Signature: \_\_\_\_\_

From: <http://www.oardc.ohio-state.edu/stockingerlab/Protocols/Electroporation.pdf>

Date and Time: \_\_\_\_\_ Performed by: ANNA GARNEY

### Ligation ~~Restriction Enzyme Digest~~

- Optimal Digestion is reached by using the correct reaction volume of your components. SO BE CAREFUL IN YOUR MEASUREMENTS
- Please be specific in where you found your materials. (For DNA, reference previous page in which it was prepared)
- Please be descriptive in your procedure in order to help clarify for others.
- Include ANY deviations from the protocol.

### PROCEDURE

#### Material Location

- o Buffer \_\_\_\_\_
- o DNA \_\_\_\_\_
- o Enzyme \_\_\_\_\_

| MATERIALS | TYPE | AMOUNT USED |
|-----------|------|-------------|
| Buffer    |      |             |
| DNA       |      |             |
| Enzyme(s) |      |             |
| Water     | DEPC |             |

Total Reaction Volume 20 $\mu$ L

Construct a table with this format for each reaction that you do today.

pBAD + RBS + cut sites + K215104 (1000)

PLAC + RBS + cut sites + K215104

Ligase Buffer 2 $\mu$ L  
 Ligase 1 $\mu$ L  
 Insert 8.5 $\mu$ L  
 Vector 8.5 $\mu$ L

Ligase Buffer 2 $\mu$ L  
 Ligase 1 $\mu$ L  
 PLAC 8.5 $\mu$ L  
 K215104 8.5 $\mu$ L

Negative 1

Ligase Buffer 2 $\mu$ L  
 Ligase 1 $\mu$ L  
 pBAD 8 $\mu$ L  
 DEPC 9 $\mu$ L

Negative 2

Ligase Buffer 2 $\mu$ L  
 Ligase 1 $\mu$ L  
 PLAC 8 $\mu$ L  
 DEPC 9 $\mu$ L

Negative 3

Ligase Buffer 2 $\mu$ L  
 Ligase 1 $\mu$ L  
 K215104 8.5 $\mu$ L  
 DEPC 8.5 $\mu$ L

Start Time of Incubation: \_\_\_\_\_

End Time of Incubation: \_\_\_\_\_

Note: incubation time is typically 1 hour

Temperature of Incubation (Determined by restriction enzymes): \_\_\_\_\_

Method used for Quenching the Reaction (*different for different enzymes*)

---

Where did you store your finished product and what did you label it?

---

|                          |
|--------------------------|
| <p><b>NEXT STEP:</b></p> |
|--------------------------|